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Full Length Research Paper

High efficiency macropropagation of potato (*Solanum tuberosum* L.) cv. Kufri Jyoti in Kumaun Hills

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The present study demonstrated the use of various PGR combinations for efficient *in vitro* regeneration of cv. kufri jyoti in kumaun hills. Best callus induction and proliferation was observed in MS medium supplemented with 13.59 µM 2,4-D alone and 2,4-D + kinetin (9.06 + 1.16 µM) out of different concentrations of 2,4-D (4.53 to 18.12 µM) alone and 2,4-D (0 to 18.12 µM) with kinetin (1.16 µM). Leaf explants were more efficient in producing callus as compared to internodes. Medium supplemented with BA + GA_3 (8.88 µM + 1 µM) initiated shoot induction out of various combinations of BA (4.44 to 13.22 µM) and GA_3 (1 µM) after 7 days of incubation with significantly high average number of shoots, average shoot length and average number of leaves per explant. MS medium supplemented with different concentrations of zeatin (4.56, 9.12 and 13.68 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) was tried for direct regeneration of shoots through nodes out of which zeatin + IAA + GA_3 (13.68 µM + 5.71 µM + 8.49 µM) served to be the best combination and the raised plantlets were found to produce microtubers in a period of 8 to 10 weeks. 2.45 µM IBA in full strength basal MS medium induced highest number of roots. In addition to an efficient regeneration protocol, the microtuber production was also studied in the present piece of work. The research protocol may also be utilized for *Agrobacterium tumefaciens* mediated transformation towards the biotic and abiotic stress tolerant potato crop.

Key words: *In vitro*, potato, callus, direct regeneration, microtubers.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most economically important tuber crop belonging to the family Solanaceae. It plays an important role in the food chain, as it ranks 4th in importance after rice, wheat and maize (Solomon and Barker, 2001). Potato is a good, cheap source of carbohydrates, vitamins, minerals and proteins. It has multipurpose use in daily consumption and also industrial purpose (Hoque, 2010). cv. Kufri jyoti is well adapted to North and South Indian hills, parts of Bihar, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Uttar Pradesh and West Bengal. It persists medium to long tuber dormancy, low storage losses and medium to high tuber dry matter. Appropriate combinations and concentrations of PGR in the culture media are required for rapid plant regeneration from explants (Ehsanpour and Jones, 2000a). *In vitro* regeneration of potato is easily done from different explants on MS medium supplemented with different PGR for diseases free good quality seeds and pathogen free planting materials (Hossain, 1994; Rabbani et

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al., 2001; Zaman et al., 2001). Successful *in vitro* plant regeneration of potato has been achieved from explants of different organs and tissues of potato such as leaf, stem, tuber discs and unripe zygotic embryos (Shirin et al*.,* 2007). The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues (Khalafalla et al., 2010**)**. Tissue culture based potato multiplication has successfully been incorporated in high quality potato seed production programme (Srivastava et al., 2012).

Microtubers (*in vitro* developed tubers) are miniature seed potatoes and they represent an intermediary phase between *in vitro* plantlets and minitubers. The use of microtubers in storage and exchange of germplasm and seed potato production is advantageous (Seabrook et al., 1993; Rannali et al., 1994). Microtubers are the first generation of potato seed from tissue culture, being used to solve the problems of transplanting the plantlets from *in vitro* to *in vivo* conditions. They can be planted directly in the soil and they can be produced in any period of the year (Nistor et al., 2010).

Considering the main problems of potato cultivation in hills of Uttarakhand including biotic and abiotic stress, lack of seed agency who provide the quality seed potato and lack of technology intervention, the present study was undertaken to develop efficient protocol for *in vitro* regeneration of cv. kufri jyoti in kumaun hills directly through nodes and via callus using leaf and internodes as explants. This protocol may serve as a highly useful technique for crop improvement through *Agrobacterium tumefaciens* mediated transformation via rapid multiplication of plantlet production as well as virus free seed potatoes or microtuber formation.

MATERIALS AND METHODS

Plant material

cv. Kufri jyoti was obtained from Government Breeding Garden, Kashipur, Uttarakhand and grown in pots (20 × 15 cm) containing soil and farmyard manure in a ratio of 3:1 over a period of 10 to 15 days. All the explants were taken from these donor plants for the present research work. Explants such as juvenile leaf, nodes and internodes were initially washed with Tween-20 and then with distilled water 3 to 4 times to remove the traces of the chemical applied. Thereafter they were treated with bavistin (fungicide) solution (0.5%, 15 min) to avoid fungal contamination. For surface sterilization explants were subjected to $HgCl_2$ (0.1%, 1 min) and thoroughly washed with distilled water for 2 to 3 times under laminar airflow. Leaves were dissected into small pieces and trimmed, nodes and internodes were cut into small pieces (approx. 5 mm). After a quick dip in 70% alcohol explants were then washed with sterile distilled water.

Preparation of culture media and growth condition

Murashige and Skoog medium (1962) was used with 3% sucrose and solidified with 0.7% agar. For root development clarigel (0.24%) was used as solidifying agent for clear analysis. Plant growth regulators used were 2,4-D, zeatin, kinetin, IAA, GA_3, BAP and IBA. All experiments were carried out in 250 ml jam bottles /flasks containing 50 ml of culture medium. The pH of media was adjusted to 5.8 using 1N NaOH prior to autoclaving at 121°C at 15 lbs pressure for 20 min. Cultures were incubated under 16 h photoperiod with photosynthetic photon flux density of 40 µ mol m^{-2} s^{-1} fluorescent lamps.

Callus induction and shoot regeneration

For callus induction juvenile leaf sections and internodes with cut ends were placed on MS medium with different concentrations of PGR like 2,4-D (4.53 to 18.12 µM) alone and 2,4-D (0 to 18.12 µM) with kinetin (1.16 µM). Callus initiated after 15 to 20 days of incubation. Calli were subcultured in every 15 days. Well differentiated calli were placed on MS medium supplemented with various combinations of BAP (4.44 to 13.22 µM) and GA_3 (1 µM) for shoot regeneration. All cultures were maintained at 25 ± 2°C with 16 h photoperiod. Shoot regeneration initiated in 7 days.

Direct regeneration of shoots

For direct regeneration of shoots explants taken were nodes. Explants were cut into small sections of 2 to 5 mm size and inoculated in the MS medium supplemented with different concentrations of zeatin (4.56, 9.12 and 13.68 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) for shoot multiplication. Cultures were kept at 25 ± 2°C with 16 h photoperiod. Shoot induction initiated in 3 to 4 days of incubation.

Regeneration of roots and development of elite plantlets

When shoots grew upto a height of 3 to 4 cm, they were aseptically removed, separated from each other and subcultured on half and full strength MS medium with varying concentrations of IBA for root induction. Root development initiated after 4 to 5 days of incubation. The completely rooted plants (2 to 3 weeks) were taken out carefully and gently washed under running water to remove excess clarigel. They were then potted in thermocole cups (12 × 8 cm) containing soil and farmyard manure (3:1, v/v); covered with transparent polythene bags with small holes to maintain humidity. These plants were placed inside growth chamber under 16 h photoperiod with photosynthetic photon flux density of 40 µ mol m^{-2} s^{-1} fluorescent lamps at 25 ± 2°C temperature. Plants were watered regularly and gradually acclimatized over a period of 1 month. The polythene bags were then removed and the established plantlets were subsequently transplanted to earthen pots (20 × 15 cm) and kept in a polyhouse for further growth (Figure 1i, j and k).

Production of microtubers

Well grown plantlets obtained from direct regeneration of nodes were maintained in culture room at 16/8 h light/dark condition and observed for the production of microtubers .

Statistical analysis

All the experimental observations were recorded at regular intervals. Mean values of various treatments were analysed by using one way ANOVA (Analysis of Variance) for statistical significance. Effect of different concentrations of plant growth

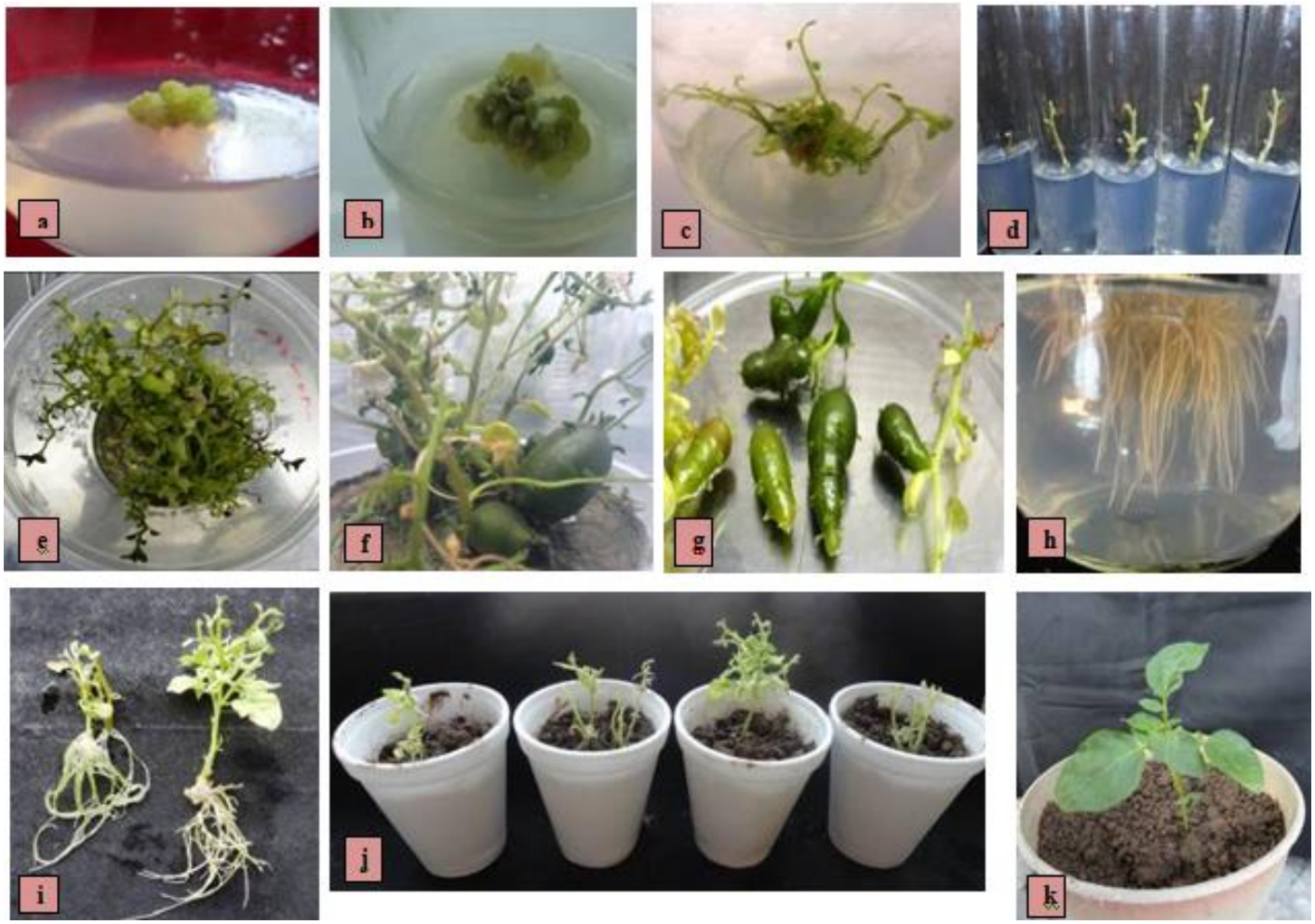

Figure 1. Different stages in macro propagation of *S. tuberosum* in full strength MS medium. (a) *In vitro* callus induction and proliferation with 2,4-D + kinetin (9.06 + 1.16 µM) after 14 of incubation;(b, c) *In vitro* shoot induction and proliferation from 8 weeks old callus with BAP (8.88 µM) + GA_3 (1.00 µM) after 7 and 14 days of incubation respectively; (d, e) *In vitro* direct shoot regeneration from nodes using different PGRs viz. zeatin + IAA + GA_3 (13.68 + 5.71 + 8.49 µM) after 7 and 49 days of incubation respectively; (f, g) Microtubers formation from *in vitro* direct shoot regenerated plantlets containing zeatin (13.18 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) under 16/8 h light/dark photoperiod after 60 days of incubation. (h) *In vitro* root induction in microshoots of *S. tuberosum* in full strength MS medium containing 2.45 µM IBA after 15 days of incubation; (i, j) *In vitro* developed plantlets were transferred to thermocole cups containing soil and farmyard manure (3:1, v/v) for acclimatization and hardening in culture room conditions for 30 days (k) *In vitro* hardened plantlets were successfully transferred to polyhouse conditions.

regulators were determined on average number of shoots, average shoot length, number of nodes and average number of leaves per explant.

RESULTS

The explants showed callus formation in MS medium containing 2,4-D. Best callus induction and proliferation was observed after 15 to 20 days of incubation in MS medium with 13.59 µM 2,4-D alone and 2,4-D + kinetin (9.06 + 1.16 µM) (Figure 1a). The callus obtained was light green in colour. Increased concentration of hormones lead to browning of callus (Tables 1 and 2). This phenomenon was also supported by previous studies in other species. Auxin alone and in combination with cytokinin can produce callus but 2,4-D was found to be most effective for callus induction and proliferation (Shirin et al., 2007). But on the contrary, in the present study callus induction in leaf explants was more frequent in comparison to internodes as explants as observed after 30 days of incubation. Regular subculture of callus enhanced proliferation rate due to availability of nutrients before their exhaustion in the medium.

Shoot regeneration from the calli initiated in the medium supplemented with BAP + GA_3 (8.88 + 1 µM) after 7 days of incubation (Figure 1b). GA_3 has been reported to help in elongation of shoots. Combination of

Table 1. Effect of different combinations of 2,4-D + kinetin in full strength MS medium on *in vitro* callus induction in various explants (leaf, internodes) of *Solanum tuberosum* after 30 days of incubation.

PGRs	Concentration (µM)	Callus growth	Callus colour
2,4-D + kinetin	0.00 + 1.16	-	-
	4.53 + 1.16	+	Light yellow
	6.79 + 1.16	+	Light yellow
	9.06 + 1.16	**+++**	Light green
	11.32 + 1.16	++	Light green
	13.59 + 1.16	++	Light green
	15.85 + 1.16	+	Light green
	18.12 + 1.16	+	Light brown

+++, Assumed as 100% response; ++, assumed as 75% response; +: assumed as 50% response; **-,** for no callus.

Table 2. Effect of different concentrations of 2,4-D in full strength MS medium on *in vitro* callus induction in various explants (leaf, internodes) of *Solanum tuberosum* after 30 days of incubation.

	Concentration (µM)	Callus growth	Callus colour
2,4-D	0.00	-	-
	4.53	-	-
	6.79	-	-
	9.06	+	Whitish
	11.33	++	Light Green
	13.59	**+++**	Light Green
	15.85	+	Light Green
	18.12	+	Light Brown

+++, Assumed as 100% response; ++, assumed as 75% response; +,: assumed as 50% response; **-,** for no callus.

Table 3. Effect of various combinations of BAP and GA_3 in full strength MS medium on *in vitro* shoot regeneration from callus of *S. tuberosum* after 30 days of incubation [values are mean ± S.E. (n=3)]

SN	Treatments	Avg. no. of shoots explant^{-1}	Avg. shoot length (cm)	Avg. no. of leaves
1	Control	0.00	0.00	0.00
2	BA + GA_3 (4.44 + 1 µM)	0.88 ± 0.11	2.49 ± 0.53	5.55 ± 1.28
3	BA + GA_3 (8.88 + 1 µM)	2.99 ± 0.19	3.48 ± 0.19	7.02 ± 0.15
4	BA + GA_3 (13.22 + 1 µM)	0.55 ± 0.11	0.82 ± 0.09	1.55 ±0.11
	LSD (P≤0.05)	7.587724*	0.000108*	0.000177*

ns, Non significant; *, significant at $p < 0.05$; S.E, standard error of mean; LSD, least significant difference; SN, serial number.

BA + GA_3 (8.88 + 1 µM) produced significantly high average number of shoots, average shoot length and average number of leaves per explant as compared to other combinations (Table 3 and Figure 1c). The results of one way analysis of variance (ANOVA) showed that F-factor and P- value for most of the parameters were significant at 0.05% level (Table 4). Longest shoot obtained was 4.50 cm in height as observed after 20 to 25 days of incubation. This agrees with Haque et al. (2009) who observed the longest shoot by the treatment combination of BAP and GA_3 in other species of the plant . On the other hand direct regeneration of shoots with highest average number of shoots, nodes and leaves per explant took place in the medium supplemented with zeatin + IAA + GA_3 (13.68 µM + 5.7 µM + 8.49 µM) (Table 5 and Figure 1d). Longest shoot attained a height

Table 4. F-ratio and level of significance of one way analysis of variance (ANOVA) test for *in vitro* shoot regeneration from callus of *S. tuberosum* after 30 days of incubation on full strength MS medium containing various combinations of BAP and GA_3.

Source of variation	Avg. no. of shoots explant^{-1}			Avg. shoot length (cm)			Avg. no. of leaves explant^{-1}		
	df	MS	F	df	MS	F	df	MS	F
Between groups	3	5.15	110.22*	3	7.44	29.82*	3	32.70	26.02*
Within Groups	8	0.04		8	0.24		8	1.25	
Total	11			11			11		

Data were recorded after every 1 week of culture. All values are an average of 9 explants; individual treatments consisted of three replicates, one explants per flask and the experiment was repeated thrice. MS, mean square; F, *f* statistic; LSD, least significant difference; df, degree of freedom, ns, not significant; *, significant at 0.05 level.

Table 5. Effect of different concentrations of zeatin, IAA and GA_3 on *in vitro* direct shoot regeneration from nodes of *S. tuberosum* on full strength MS medium after 21 days of incubation [values are mean ± S.E (n=3)].

S/N	Treatments	Avg. no. of shoots explant^{-1}	Avg. Shoot length (cm)	Avg. no. of nodes explant^{-1}	Avg. no. of leaves explant^{-1}
1	Control	1.00 ± 0.00	1.12 ± 0.12	0.00	1.00 ± 0.40
2	Zeatin+IAA+GA_3 (4.56+5.71+ 8.49 µM)	0.99 ± 0.13	1.35 ± 0.29	0.91 ± 0.39	3.87 ± 0.95
3	Zeatin+IAA+GA_3 (9.12+5.71+ 8.49 µM)	1.41 ± 0.08	3.17 ± 0.34	2.10 ± 0.39	8.55 ± 1.47
4	Zeatin+IAA+GA_3 (13.68+5.71+ 8.49 µM)	1.49 ± 0.21	4.10 ± 0.27	2.90 ± 0.29	12.37 ± 1.90
LSD (P≤0.05)		0.036155*	0.000011*	0.000110*	0.000320*

ns, Non significant; *, significant at p < 0.05; S.E., standard error of mean; SN, serial number.

Table 6. F-ratio and level of significance of one way analysis of variance (ANOVA) test for *in vitro* direct shoot regeneration from nodes of *S. tuberosum* on full strength MS medium containing different concentrations of zeatin, IAA and GA_3 after 21 days of incubation.

Source of variation	Avg. no. of shoots explant^{-1}			Avg. Shoot length			Avg. No. of nodes explant^{-1}			Avg. no. of leaves explant^{-1}		
	df	*MS*	*F*	*df*	*MS*	*F*	*df*	*MS*	*F*	*df*	*MS*	*F*
Between groups	3	0.28	3.93*	3	8.28	27.32*	3	6.93	17.52*	3	101.10	13.96*
Within groups	12	0.07		12	0.30		12	0.39		12	7.23	
Total	15			15			15			15		

Data were recorded after every 1 week of culture. All values are an average of 12 explants; individual treatments consisted of four replicates, one explants per flask and the experiment was repeated thrice with qualitatively similar results. MS, Mean square; F, *f* statistic; LSD, least-significant difference; df, degree of freedom, ns, not significant; *, significant at 0.05 level. S.E, Standard error of mean.

of 6 cm after 15 to 20 days of incubation. Profound shoot proliferation was obtained after 7 to 8 weeks (Figure 1e). Direct shoot regeneration is preferred since it reduces the possibility of somaclonal variation (genetic variation) common in plants regenerated from cultured cells or tissues (Misra and Datta, 2001; Dayal et al., 2003). Results of this experiment are also proved to be significant using ANOVA (Table 6). Observations recorded were observed for different parameters viz. average number of shoots, average shoot length, number of nodes and average number of leaves per explant.

Plantlets were found to produce microtubers in a period of 8 to 10 weeks (Figure 1f and g). Cytokinins are believed to have strong promotive effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Pelacho and Mingo-Castel, 1991). The average weight of the tubers obtained was found to be 0.20 g with an average number of 3 tubers per explant. The microtubers obtained were green in colour. For root induction, out of different concentrations of IBA tried with basal MS medium and half strength basal MS medium, 2.45 µM IBA induced highest number of roots after 15 days of incubation in full strength basal MS medium (Figure 1h) as well as in half strength basal MS medium as compared to other concentrations. IBA has proved to be efficient in promoting root induction (Sakthivel and Manigandan, 2011). The mean value of *in vitro* rooting

Table 7. Various parameters studied under the effect of different concentrations of IBA in half strength and full strength MS medium on rooting response in *in vitro* multiplied shoots of *S. tuberosum* after 15 days of incubation [values are mean ± S.E (n=3)]

S/N	Treatment	Avg. no. of roots shoot^{-1}	Avg. root length (cm)
	Half strength medium		
1	IBA (1.12 µM)	37.50 ± 12.50	7.50 ± 0.50
2	IBA (2.45 µM)	42.40 ± 7.50	7.25 ± 0.50
3	IBA (4.9 µM)	37.50 ± 2.50	7.50 ± 0.50
	Full strength medium		
4	IBA (1.12 µM)	34.00 ± 4.00	7.50 ± 0.00
5	IBA (2.45 µM)	43.50 ± 1.50	7.50 ± 0.50
6	IBA (4.9 µM)	31.50 ± 1.50	7.25 ± 0.25

S.E., Standard error of mean; SN, serial number.

response for all the parameters at different PGR concentration showed that average number of root (43.50) and average root length (7.50 cm) was observed to be maximum with 2.45 µM IBA in full strength MS medium. Longest root attained the length of 8.5 cm. Data analysed for average number of roots and average root length for different concentrations of IBA used is represented in Table 7.

DISCUSSION

Callus is an unorganized mass of plant cells. Reliable callus induction and regeneration of viable plants is considered as a limiting step to the successful use of modern techniques in genetic improvement of the major crops (Murphy, 2003). In the present study, different PGR combinations were checked for *in vitro* callus induction in explants of *Solanum tuberosum*. Callus induction was found to be successful using different concentrations of 2,4-D alone and in combination with Kinetin. The auxin 2,4-D, by itself or in combination with cytokinins, has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4-D as the best auxin for callus induction both in monocots and dicots (Chee, 1990; Mamun et al., 1996). Role of 2,4-D in callus induction has been widely accepted and utilized for potato, tomato and many medicinal plants (Ashakiran et al., 2011; Ahmed et al., 2012; Lakshmi and Reddy, 2012; Mehta et al., 2012).

On the basis of regular observation it was concluded that the source of explant has a direct effect on callus induction. Results showed that leaf explant were more efficient for callus induction with 100% response as compared to internodes which gave only 50% response. This may be due to the presence of more meristematic activity in leaves as compared to internodes. This result supported the previous study by Haque et al. (2009) who found that callus length was affected by different explants and that the leaf explants produced significantly highest callus length in contrast to the shoot tip which produced least results in case of potato cv. Diamant. The interaction effect between explant and concentration of growth regulators were found to have significant differences on callus length in different researches. This result was also proved to be significant by Dobranaszki et al. (1999) and Fomenko et al. (1998) who also observed significant effects of explants of potato on callus length. In the present study different concentrations of 2,4-D and Kinetin showed significant differences in callus growth and colour. Rate of callus induction increased with the increasing concentration of 2,4-D alone upto 13.59 µm and in the combination of 2,4-D and Kinetin upto 9.06 µm and 1.16 µm respectively. Further increase in concentration lead to decrease in callus growth and resulted in browning of callus. Callus initiation on cut ends of *in vitro* cultured explants of potato could be observed in all 2,4-D levels (Khalafalla et al., 2010). Similar findings were also reported by (Fiegert et al., 2000; Jayasree et al., 2001; Yasmin et al., 2003).

Both callus induction and plant regeneration from explant require appropriate combinations and concentrations of plant growth regulators in the culture media (Ehsanpour et al., 2000b). In the present research work, best results for shoot regeneration from callus of *S. tuberosum* was obtained by using a combination of 8.88 µM BAP and 1.00 µM GA_3 with significantly high average number of shoots, shoot length and number of leaves per explant as compared to other combinations. BAP, Zeatin or Kinetin are known to help produce adventitious shoots. Martel and Carcia (1992) reported that both BAP and GA_3 at higher concentrations were necessary for shoot formation of potato. Shoot regeneration responses vary with the potato cultivar but in most cases cytokinin helps to enhance shoot production (Ghaffoor et al., 2003). Generally a low ratio of auxin to cytokinin is required for adventitious shoot development in case of potato (Anjum and Ali, 2004).

A decrease in all the parameters of shoot regeneration occurred after increase in the concentration of BAP after 8.88 µM. Similar effects of increasing concentration of BAP on shoot regeneration of potato cv. Asterix were observed by Molla et al. (2011) who observed that the length of shoot increased with increasing BAP concentration up to 3 mg l^{-1} and then decreased. Role of GA_3 in shoot elongation is well known and reported by many researchers. For rapid multiplication, addition of GA_3 to the MS media has been reported to improve growth and development of shoots. Farhatullah and Abbas (2007) also have reported that dosage of 0.248 mg l^{-1} of GA_3 in the MS medium boosted all morphological characters in *in vitro* raised potato plantlets. Ullah et al. (2012) also have reported that GA_3 is involved in cell elongation and its addition in MS medium enhanced shoot growth in *in vitro* propagated plants of potato variety "Desiree".

Direct regeneration system has an edge over regeneration after passing through callus phase to maintain the true-to-type nature of the regenerated plantlets and avoid somaclonal variation. Potato breeding programs can highly benefit from biotechnological tools, which are capable of surpassing some limitations found by traditional plant breeding methods and open new avenues for crop improvement. In the present study, attempts were made also made to induce direct regeneration of *S. tuberosum.* Explant used were nodes. Leaf discs and inter nodal tissues are the least responsive explants for direct regeneration. These explants underwent callus induction phase and then resulted in shoot regeneration indirectly in a study conducted by Hussain et al. (2005) on three potato cultivars viz., Cardinal, Altamash and Diamont. There are many advantages of taking nodal tissue as an explant, that is, a large number of aseptic plants can be obtained quickly and easily, and plants produced may remain true to type. Successful regeneration was obtained using hormonal combination of Zeatin, IAA and GA_3 in a concentration of 13.68, 5.71 and 8.49 µM, respectively. Role of Zeatin in regeneration has been reported by Wendt et al. (2001) who found that the internode explant of potato cultivar Macaca treated with Zeatin showed higher regeneration rate than those treated with BAP.

Roots were induced in microshoots using different concentrations of IBA, out of which 2.45 µM concentration emerged to be best with maximum average number of root (43.50) and a maximum average root length of 7.50 cm in full strength MS medium. IBA has been shown as a potent root inducer in many studies conducted on various tomato cultivars (Chaudhry et al., 2010; Khalafalla et al., 2010; Sakthivel and Manigandan, 2011).

Microtubers of *S. tuberosum* were obtained after incubating directly regenerated shoots at 16/8 h light/dark condition after 8 to 10 weeks. Microtubers obtained were green in colour. The green colour might be due to the presence of alkaloid solanin which is produced under light conditions. Microtubers may vary in their shape, colour, weight, diameter, length etc. (Rannali, 2007). This study also supports the similar findings of Hoque (2010). The edible part of the plant is the tuber, which is formed at the end of underground stems called stolon. Potato produced more protein and calories per unit area per unit time and per unit of water than any other major plant food. *In vitro* tubers can be produced throughout the year and thus holds benefit over conventional tubers (Hoque, 2010).

Conclusion

The present regeneration protocol could be useful for rapid *in vitro* regeneration, multiplication and virus free seed, that is, microtuber production. This piece of work may also be utilized for transformation techniques for production of biotic and abiotic stress tolerant potato crop which may in turn contribute to overcome major obstacle in potato farming especially in the Kumaun hills towards quality and efficient production of this major cash crop.

Conflict of Interest

The authors have not declared any conflict of interest.

Abbreviations: ANOVA, analysis of variance; **BA,** benzyl adenine; **GA_3,** gibberellic acid; **IAA,** indole acetic acid; **MS,** Murashige and Skoog (1962); **PGR,** plant growth regulators; **2,4-D,** 2,4-dichlorophenoxy acetic acid.

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Identification of sorghum (*Sorghum bicolor* L. Moench) landraces tolerant to post flowering drought stress using drought tolerance indices

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Drought stress occurring during the post-flowering growth stage of sorghum can cause considerable reduction in yield. In order to identify drought tolerant Eritrean sorghum landraces and assess efficiency of drought tolerance indices, twenty five sorghum (*Sorghum bicolor* L. Moench) accessions were evaluated in split plot design with three replications. Fully irrigated and drought stress treatments were assigned in main plot and the landraces were evaluated in sub plot for drought stress tolerance at post-flowering. Seven tolerance indices including stress tolerance index (STI), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), tolerance index (TOL), yield index (YI), and yield stability index (YSI) were estimated for each genotype based on grain yield under drought stress (Y_s) and irrigated conditions (Y_{ir}). Significant correlations between Y_{ir} and Y_s with GMP, MP, STI and YI were recorded indicating that these indices were good predictors of drought tolerance among genotypes. The other stress tolerance indices namely, TOL, SSI, YSI and YI were not significantly correlated with Y_{ir} and Y_s indicating that they were poor predictors of drought tolerance. The study further showed that drought stress reduced the yield of some genotypes while others were tolerant to drought and gave stable yield. Based on the tolerance indices, accessions EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711 were identified as superior genotypes for post-flowering drought tolerance that could be used by breeders in further sorghum improvement programs.

Key words: Drought stress, drought tolerance, post-flowering, selection index, *Sorghum bicolor.*

INTRODUCTION

The improvement of drought tolerance has been defined as a desirable breeding objective in crops (Clark et al., 1992). Drought tolerance in native plant species is often defined as survival, but in crop species it is defined in terms of productivity (Passioura, 1983). The definition of drought tolerance as the ability of plants to grow

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satisfactorily when exposed to water deficits has little direct applicability to either quantifying or breeding for the character in crop species (Clark et al., 1992). Generally, it is agreed that drought tolerance from a breeding viewpoint is a complex trait that shows a high level of genotype × environment interaction (Cooper et al., 2006). Furthermore, plant responses to drought are also influenced by the time, intensity, duration, and frequency of the stress as well as by diverse plant–soil–atmosphere interactions (Saint Pierre et al., 2012). However, for studies on adaptation of crop plants to complex stress situations arising due to climate change, there is a need to exploit the available biodiversity in crop genotypes growing in diverse environments to understand the mechanisms involved in coping with different stress combinations. Accordingly, genotypes that differ in drought tolerance serve as important systems for studying adaptive responses to drought in crop species (Bhargava and Sawant, 2013). Drought stress affects almost every developmental stage of the plant. However, damaging effects of this stress is more noted when it coincides with various growth stages such as germination; seedling shoot length, root length and flowering (Rauf, 2008; Khayatnezhad et al., 2010).

Several morpho-physiological characteristics have been reported as reliable indicators in selection of genotypes/cultivars for drought tolerance. Information about morpho-physiological traits and the gene effects controlling the highly related traits to drought tolerance makes breeding programs for drought tolerance much more effective and successful (Badieh et al., 2012). A range of stress tolerance indices including yield, morphological, and physiological traits has been suggested that could be utilized to increase selection efficiency and can be used for screening tolerant genotypes under stress conditions (Drikvand et al., 2012). However, yield is the principle selection index used commonly under drought stress conditions. Furthermore, correlation analysis between grain yield and drought tolerance indices can be a good criterion for screening the best genotypes and indices used (Farshadfar et al., 2012). Farshadfar et al. (2001) reported that the most appropriate index for selecting stress tolerant cultivars is an index which has high correlation with seed yield under stress and non-stress conditions. Yield-based estimates of drought tolerance are as follows: geometric mean productivity (GMP) which was proposed to select genotypes based on their performance in stress and non-stress environments (Fernandez, 1992). Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the drought stress and irrigated environments and mean productivity (MP) as the average yield of genotypes under irrigated (Y_{ir}) and drought stress (Y_s) conditions. Fischer and Maurer (1978) proposed a stress susceptibility index (SSI). Fernandez (1992) stated that stress tolerance index (STI) can be used to identify genotypes that produce high yield under both stressed and non-stressed conditions. Screening and selection of plants of different crops with considerable drought stress tolerance at flowering and post-flowering stage has been considered as an economic and efficient means of utilizing drought-prone areas when combined with appropriate management practices to reduce water loss (Rehman et al., 2005). The objective of this study was therefore to identify drought tolerant sorghum landraces for cultivation in drought-prone areas of Eritrea using stress tolerance indices.

MATERIALS AND METHODS

Germplasm

The germplasm used in this study comprised 25 sorghum genotypes including 23 accessions from the Eritrean sorghum gene bank and two improved (B-35 and Hamelmalo) from ICRISAT and National Breeding Program, respectively (Table 1).

Location

The experiment was conducted under managed irrigated and stress condition at Hamelmalo Agricultural College (HAC) farm in 2014 dry season period in the months of February to June. Geographically the trial site is located at 15° 52'15" N latitude and 38° 27' 55" E longitudes with an altitude of 1,274 m above sea level in a semi-arid agro-ecological zone of Eritrea. The research area is located 12 km away from Keren city towards the north on the Keren-Nakfa road along Anseba River in the Anseba region. The soil type of the experimental site was sandy clay loam with an average maximum and minimum air temperatures during the experimental period reached 38 and 20°C, respectively.

Experimental design and data analysis

Split plot design was used by setting two main plots, fully irrigated and stress plots with three replications planted on 12 February, 2014. The two levels of irrigation treatments including: Full irrigation (fully irrigated based on plant needs of sorghum accessions at different growth stages) and Limited irrigation (Supply plant water needs until flowering stage and then format water until the end of sorghum growth and development).

The spacing between the irrigated and stressed replications was 3 m. The sub plots were the 25 genotypes that were planted in plots of four rows with a spacing of 75 cm x 20 cm between and within rows, respectively and three meter row length. Soil moisture content before sowing, during and after imposing stress (at flowering growth stage) was taken by the department of Land Resources and Environment of Hamelmalo Agricultural College. For determining the final yield, the panicles of the two middle rows of an area of 4.5 m^2 (2 rows of 3 m long) were harvested at maturity and yields recorded that was used for the analysis. Stress tolerance indices were used to identify germplasm accessions with high stress tolerance and overall good agronomic performances. The drought stress indices were calculated according to Agili et al. (2012) as follows:

*Stress Susceptibility Index (SSI) = $[1-(Y_s/Y_{ir})]/SI$, Where $SI = 1- (\overline{Y}_s/\overline{Y}_{ir})$

*****Mean Productivity (MP) = $(Y_{ir} + Y_s)/2$

Table 1. Twenty five sorghum accessions along with their sources, names and status.

S/N	Germplasm identifier	Area of collection (administration region)	Local Name	Status
1	EG 469	Gash Barka	Tseda Bazenay	Landrace
2	EG 849	Gash Barka	Hugurtay	Landrace
3	EG 537	South	Anseba	Landrace
4	Hamelmalo	Anseba and Gash Barka	Hamelmalo	Released cultivar
5	EG 806	Gash Barka	Hiriray	Landrace
6	EG 782	South	Tseda Hele	Landrace
7	EG 797	Gash Barka	Wedi-Aker	Landrace
8	EG 791	Gash Barka	Korekora	Landrace
9	EG 815	Gash Barka	Estif	Landrace
10	EG 836	Anseba	Hugurtay	Landrace
11	EG 883	Gash Barka	Kinabiba	Landrace
12	EG 885	Gash Barka	Duruta	Landrace
13	EG 889	Gash Barka	Kileaentu	Landrace
14	EG 1224	Gash Barka	Mahagen	Landrace
15	EG 526	Anseba	Wedi-Aker (Short)	Landrace
16	EG 711	Anseba	Embulbul	Landrace
17	EG 783	Gash Barka	Aklamoy	Landrace
18	EG 813	Anseba	Wedi-Ferej	Landrace
19	EG 830	Gash Barka	Wedi-Arba	Landrace
20	EG 481	Anseba	Wedi-Susa	Landrace
21	H-35-1	South	Tseda Mashela	Landrace
22	B-35	ICRISAT	B-35	Released cultivar
23	EG 870	Gash Barka	Ajebsidu	Landrace
24	EG 473	South	Keih Hele	Landrace
25	EG 843	South	Koden	Landrace

*Tolerance (TOL) = Y_{ir}-Y_s

*Stress Tolerance Index $(STI) = Y_{ir} * Y_s / \overline{Y}_{ir}^2$

*Geometric Mean Productivity $(GMP) = \sqrt{Y_{ir} * Y_s}$

*Yield Index $(YI) = Y_s / \overline{Y}_s$

*Yield Stability Index (YSI) = Y_s / Y_{ir}

Where:
*Y_{ir} = Yield of accessions in normal irrigation conditions
*Y_s = Yield of accessions in drought stress conditions
*$\overline{Y}_{ir}$ = Mean yield in normal irrigation conditions
*$\overline{Y}_s$ = Mean yield in drought stressed conditions

Data collection

After harvesting of the panicles from the two inner rows with a net plot area of 4.5 m^2 were dried, threshed and weighed for final yield data collection which was then converted into g m^{-2}. Analysis of variance was calculated for individual and combine treatments. Besides, the most desirable drought tolerance measures, the correlation coefficient between Y_{ir}, Y_s, and other quantitative indices of drought tolerance were estimated using GenStat 14 statistical software (Payne et al., 2011). Ranking for the drought indices were estimated by taking the sum total of individual drought indices and calculated as a mean. The lowest mean was considered maximum response while highest score was minimum response to drought tolerance. Multivariate analysis for biplot and cluster analysis were also carried out using this Genstat software to identify and classify genotypes under both stress and non-stress conditions.

RESULTS

There was a significant difference among normal irrigated and drought stressed conditions for grain yield at 1% probability level. The genotypes were also showed significant differences in grain yield at 0.1% probability level (Table 2). Grain yield varied from a high yield of 334.0 g m^{-2} (EG 849) to a low yield of 138.6 g m^{-2} (B 35) under normal irrigation conditions and from 285.8 g m^{-2} (EG 885) to 77 g m^{-2} (EG 815) in drought stress conditions. The mean combine grain yield under normal irrigation condition was 240.9 g m^{-2}, while under drought stress conditions it was 211.7 g m^{-2}, thus indicating a reduction of 12.2% compared to full-irrigation conditions (Table 3). The data showed that drought stress in sorghum can noticeably reduce the grain yield.

Table 2. Mean squares from the analysis of variance for grain yield of 25 sorghum genotypes evaluated under normal irrigation (Y_{ir}) and drought stress (Y_s) conditions at the Hamelmalo Agricultural College non rainy seasons of 2014.

Source of variation	Degree of freedom	Grain yield (MS)
Replication	2	5953.2
Irrigation level	1	32215.4**
Accessions	24	17018.1 ***
Irr. x acc.	24	1929.3
Error	96	3150.4
CV (%)		18.0

CV (%) = Coefficient of variance and Fprob. = F probability differences at ** P ≤ 0.01 and *** P ≤ 0.001.

The accessions EG 849, EG 836, EG 481, EG 883, EG 885, EG 783 and EG 469 showed higher grain yield under irrigated conditions, with yield averages higher than 290 g m^{-2}. Accessions EG 885, EG 481, EG 836, EG 469, EG 883, EG 783 and Hamelmalo recorded higher grain yield under stress conditions, with values as high as 260 g m^{-2}. The genotypes EG 481, EG 836, EG 885, EG 883 and EG 469 showed better yield performance under both irrigated and drought stressed conditions when compared with other genotypes (Table 3).

The values of geometric mean productivity (GMP) ranged from 121.6 to 298.9 g m^{-2} and the genotypes EG 836 and EG 885 were the most productive (>296 g m^{-2}). Stability tolerance index (STI) ranged from 0.26-1.54. Values ≥ 1 indicate high stress tolerance, (Majid et al., 2010). Genotypes EG 849, EG 836, EG 885, EG 481, EG 883, EG 783 and EG 469 had higher values of ≥1.35, suggesting that these genotypes were the most tolerant (Table 3). YI ranged from 0.36 to 1.35, with genotypes EG 885, EG 836, EG 481, EG 883 and EG 783 with the higher index (≥1.23). Based on YI index, the same genotypes were selected, correlated in the maximum degree with Y_s (r = 1.00) and moderately with Y_{ir} (r almost 0.79). SSI values varied from -2.49 to 5.05, which were negatively correlated with yield under drought stress (Y_s) and positively associated with the TOL index. YSI ranged from 0.39-1.30 (a higher rate indicated greater stability). Genotypes that showed higher stability indices include EG 843, B-35 and EG 791 whose values were equal or greater than 1.13 (Table 3).

Besides the mean productivity (MP) and geometric mean productivity (GMP) showed similar ranking pattern as in STI. In both indices, the top five genotypes with highest value of MP and GMP were EG 836, EG 885, EG 481, EG 883 and EG 849. Similarly, genotypes B-35, SG 843, EG 791 and Hamelmalo that showed lower SSI values also scored higher yield stability index (YSI) whereas yield index (YI) have almost similar ranking with STI values.

The indices GMP, MP and STI were very similar to the selection based on Y_{ir} and Y_s. This was confirmed by the high correlations between Y_{ir} and GMP (r = 0.94), MP (r = 0.95), and STI (r = 0.95) and the correlation between Ys and GMP (r = 0.96), MP (r = 0.94) and STI (r = 0.93) (Table 4). MP is the mean production under both stress and non-stress conditions, and it was highly correlated with yield under both conditions. Thus, MP can be used to identify cultivars in the tolerant group. Similar to the SSI and TOL, correlations between YSI and GMP, STI and MP were low (r = 0.10, r = 0.05 and r = 0.06 respectively), indicating that similar genotypes were not selected. The correlation between STI and GMP was nearly one and these two were positively correlated with MP but not with SSI. SSI was found to be highly negatively correlated with YSI and positively with TOL (Table 4).

In the biplot a strong negative association was observed between SSI and TOL with YSI, as indicated by the large angles between their vectors. Nearly zero correlation was also recorded between SSI with GMP, MP, and STI, as well as SSI and TOL with Y_s and YI, as indicated by the nearly perpendicular vectors. Besides, positive association between Y_{ir} and Y_s with MP, GMP and STI was observed as indicated by the acute angles (Figure 1). The results obtained from the biplot graph confirmed similarity with the correlation analysis results in Table 4. Thus the same as the correlation analysis the biplot was able to identify superior genotypes for both drought stressed and normally irrigated conditions.

The results of the Dendrogram from UPGMA cluster analysis (Figure 2) were consistent with those of biplot analysis (Figure 1). The advantage of this approach is that it can be used to calculate distances between genotypes. The Cluster analysis showed that the genotypes, based on TOL, MP, GMP, SSI, YI, STI and YSI, tended to group into five clusters. In this analysis, the first group (A) had the highest MP, GMP and STI, and was thus considered to be the most desirable cluster for both growth conditions. The clusters grouped in D and E

Table 3. Mean values of yield in stressed (Y_s), yield in irrigated (Y_{ir}), tolerance index (**TOL**), mean productivity (**MP**), stress susceptibility index (**SSI**), geometric mean productivity (**GMP**), stress tolerance index (**STI**), yield index (**YI**) and yield stability index (**YSI**) in sorghum

Accessions	Y_{ir} (g m^{-2})	Y_s (g m^{-2})	TOL	MP	SSI	GMP	STI	YI	YSI	Ranking
EG 849	334.00 (**1**)	238.60 (**9**)	95.40 (**2**)	286.30 (**5**)	2.35 (**3**)	282.30 (**5**)	1.47 (**3**)	1.13 (**9**)	0.71 (**23**)	5
EG 836	329.30 (**2**)	271.30 (**3**)	58.00 (**4**)	300.30 (**1**)	1.45 (**7**)	298.90 (**1**)	1.54 (**1**)	1.28 (**2**)	0.82 (**19**)	1
EG 481	313.90 (**3**)	271.60 (**2**)	42.30 (**10**)	292.80 (**3**)	1.11 (**11**)	292.00 (**3**)	1.47 (**4**)	1.28 (**3**)	0.87 (**15**)	2
EG 883	309.10 (**4**)	263.60 (**5**)	45.50 (**6**)	286.40 (**4**)	1.21 (**9**)	285.40 (**4**)	1.40 (**5**)	1.25 (**5**)	0.85 (**17**)	4
EG 885	307.00 (**5**)	285.80 (**1**)	21.20 (**16**)	296.40 (**2**)	0.57 (**18**)	296.20 (**2**)	1.51 (**2**)	1.35 (**1**)	0.93 (**8**)	3
EG 783	303.80 (**6**)	260.50 (**6**)	43.30 (**8**)	282.20 (**6**)	1.17 (**10**)	281.30 (**6**)	1.36 (**6**)	1.23 (**6**)	0.86 (**16**)	6
EG 469	292.70 (**7**)	267.10 (**4**)	25.60 (**13**)	279.90 (**7**)	0.72 (**16**)	279.60 (**7**)	1.35 (**7**)	1.26 (**4**)	0.91 (**9**)	7
EG 711	289.90 (**8**)	259.30 (**8**)	30.60 (**12**)	274.60 (**8**)	0.87 (**15**)	274.20 (**8**)	1.29 (**8**)	1.22 (**8**)	0.89 (**11**)	8
EG 813	259.00 (**9**)	199.60 (**17**)	59.40 (**3**)	229.30 (**13**)	1.89 (**5**)	227.40 (**13**)	0.89 (**13**)	0.94 (**17**)	0.77 (**21**)	11
Hamelmalo	250.20 (**10**)	260.10 (**7**)	-9.90 (**22**)	255.20 (**9**)	-0.33 (**22**)	255.10 (**9**)	1.12 (**9**)	1.23 (**7**)	1.04 (**4**)	9
B35-1	247.90 (**11**)	211.00 (**15**)	36.90 (**11**)	229.50 (**12**)	1.22 (**8**)	228.70 (**12**)	0.90 (**12**)	1.00 (**15**)	0.85 (**18**)	13
EG 830	243.00 (**12**)	222.10 (**12**)	20.90 (**18**)	232.60 (**11**)	0.71 (**17**)	232.30 (**11**)	0.93 (**11**)	1.05 (**12**)	0.91 (**10**)	12
EG 806	242.80 (**13**)	233.30 (**10**)	9.50 (**19**)	238.10 (**10**)	0.32 (**19**)	238.00 (**10**)	0.98 (**10**)	1.10 (**10**)	0.96 (**7**)	10
EG 473	227.40 (**14**)	201.90 (**16**)	25.50 (**14**)	214.70 (**17**)	0.92 (**13**)	214.30 (**17**)	0.79 (**17**)	0.95 (**16**)	0.89 (**12**)	15
EG 526	226.90 (**15**)	220.70 (**13**)	6.20 (**20**)	223.80 (**14**)	0.22 (**20**)	223.80 (**14**)	0.86 (**14**)	1.04 (**13**)	0.97 (**6**)	14
EG 537	215.60 (**16**)	215.70 (**14**)	-0.10 (**21**)	215.70 (**16**)	0.01 (**21**)	215.60 (**16**)	0.80 (**16**)	1.02 (**14**)	1.00 (**5**)	17
EG 797	215.00 (**17**)	158.30 (**21**)	56.70 (**5**)	186.70 (**19**)	2.17 (**4**)	184.50 (**19**)	0.59 (**19**)	0.75 (**21**)	0.74 (**22**)	18
EG 782	205.50 (**18**)	182.60 (**19**)	22.90 (**15**)	194.10 (**18**)	0.92 (**14**)	193.70 (**18**)	0.65 (**18**)	0.86 (**19**)	0.89 (**13**)	19
EG 791	204.50 (**19**)	230.90 (**11**)	-26.40 (**24**)	217.70 (**15**)	-1.06 (**23**)	217.30 (**15**)	0.81 (**15**)	1.09 (**11**)	1.13 (**3**)	16
EG 815	199.20 (**20**)	77.00 (**25**)	122.20 (**1**)	138.10 (**24**)	5.05 (**1**)	123.80 (**24**)	0.26 (**24**)	0.36 (**25**)	0.39 (**25**)	21
EG 889	197.00 (**21**)	154.00 (**23**)	43.00 (**9**)	175.50 (**20**)	1.80 (**6**)	174.20 (**20**)	0.52 (**20**)	0.73 (**23**)	0.78 (**20**)	20
EG 870	185.10 (**22**)	164.00 (**20**)	21.10 (**17**)	174.60 (**21**)	0.94 (**12**)	174.20 (**21**)	0.52 (**21**)	0.77 (**20**)	0.89 (**14**)	22
EG 1224	146.10 (**23**)	101.20 (**24**)	44.90 (**7**)	123.70 (**25**)	2.53 (**2**)	121.60 (**25**)	0.25 (**25**)	0.48 (**24**)	0.69 (**24**)	24
EG 843	140.80 (**24**)	183.40 (**18**)	-42.60 (**25**)	162.10 (**22**)	-2.49 (**25**)	160.70 (**22**)	0.44 (**22**)	0.87 (**18**)	1.30 (**1**)	23
B-35	138.60 (**25**)	158.20 (**22**)	-19.60 (**23**)	148.40 (**23**)	-1.16 (**24**)	148.10 (**23**)	0.38 (**23**)	0.75 (**22**)	1.14 (**2**)	25
Mean	**240.97**	**211.67**	**29.30**	**226.35**	**0.92**	**224.93**	**0.92**	**1.00**	**0.89**	

Number in brackets are ranking for the drought indices. 1: maximum response, 25: minimum response.

had lower yield and were susceptible to drought.

DISCUSSION

Genotypic correlation coefficient between Y_{ir}, Y_s and the other quantitative indices were the most desirable drought tolerance criteria to determine the performance of sorghum landraces. The strong positive association of the yield under well irrigation (Y_{ir}) with the yield under stress (Y_s) conditions depicted that genotypes giving high yield under the best possible conditions could also do so under stress conditions. This means that genotypes under drought stressed conditions have a good response under irrigated conditions. The accessions that give superior yield in both irrigated and drought stressed treatment

Table 4. Genotypic correlation of yield in normal irrigated (Y_{ir}) and stressed (Y_s) conditions with tolerance index (TOL), mean productivity (MP), stress susceptibility index (SSI), geometric mean productivity (GMP), stress tolerance index (STI), yield stability index (YSI) and yield index (YI) in sorghum.

Variables	Y_{ir}	Y_s	YSI	MP	GMP	TOL	SSI	STI	YI
Y_{ir}	1.00								
Y_s	0.798 ***	1.00							
YSI	-0.233	0.382*	1.00						
MP	0.952***	0.945***	0.068	1.00					
GMP	0.939***	0.956***	0.103	0.999***	1.00				
TOL	0.410	-0.222	-0.956***	0.111	0.073	1.00			
SSI	0.233	-0.382	-1.000***	-0.068	-0.103	0.956***	1.00		
STI	0.951***	0.935***	0.053	0.995***	0.993***	0.125	-0.053	1.00	
YI	0.798***	1.000***	0.382*	0.945***	0.956***	-0.222	-0.382*	0.935***	1.00

F probability at * P≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 significant level of probability

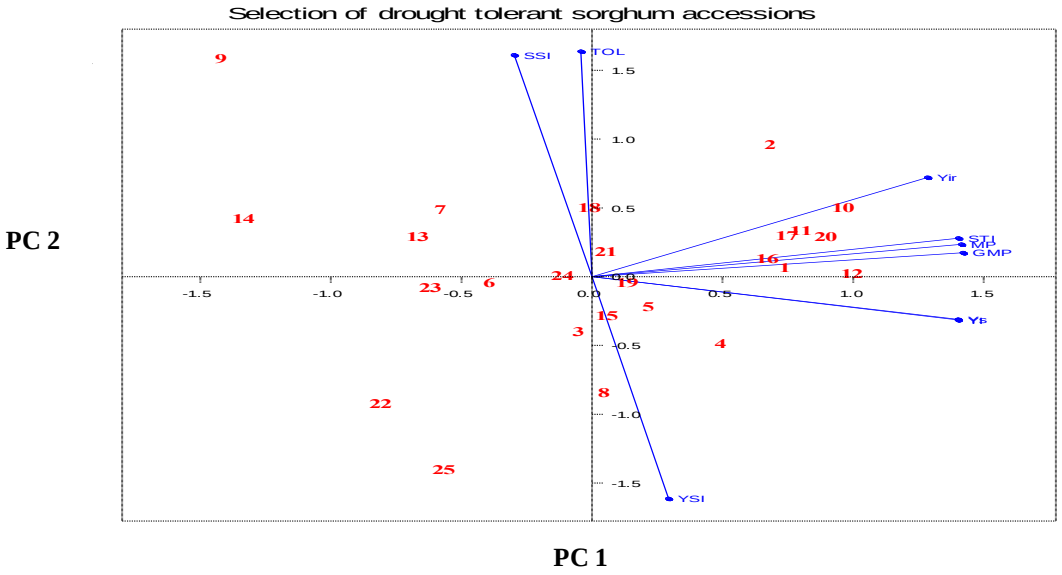

Figure 1. Biplot diagram of 25 sorghum genotypes and 7 drought indices. The indices are indicated using uppercase letters (GMP, MP, STI, YI, TOL, YSI and SSI), and each genotype is represented with numbers (see Table 1 for genotype coding).

conditions include EG 885, EG 469, EG 836, EG 481, and EG 883 as examples of high yielding genotypes. However, there were few accessions like EG 537, Hamelmalo, EG 791 and B-35 that gave better yield under stress condition only and accessions EG 836, EG 481, EG 849 and EG 813 gave superior yield under well irrigation indicated that they were the better predictors of potential yield under stress and normal irrigation respectively.

STI, GMP and MP were strongly correlated with yield under both conditions, suggesting that these parameters are suitable for screening drought tolerant and high yielding genotypes in both drought stressed and irrigated conditions. Similar results were reported by Fernandez (1992) on mung bean (*Vigna radiate*) (for STI), Agili et al. (2012) on sweet potato (*Ipomoea batatas*), Farshadfar and Sutka (2002) on wheat (*Triticum aestivum*), Golabadi et al. (2006) on durum wheat (*Triticum durum*), Sio Se-Mardeh et al. (2006) and Mohammadi et al. (2010) on wheat (*Triticum aestivum*), all of whom found these parameters to be suitable for discriminating the best genotypes under drought stress and irrigated conditions. STI was significantly correlated with Y_{ir} and Y_s and calculated based on the GMP index. High positive correlation was observed between this index (0.993), which is in agreement with those reported by Fernandez (1992) and Mozaffari et al. (1996). TOL appears to be useful for selecting genotypes with high yield under

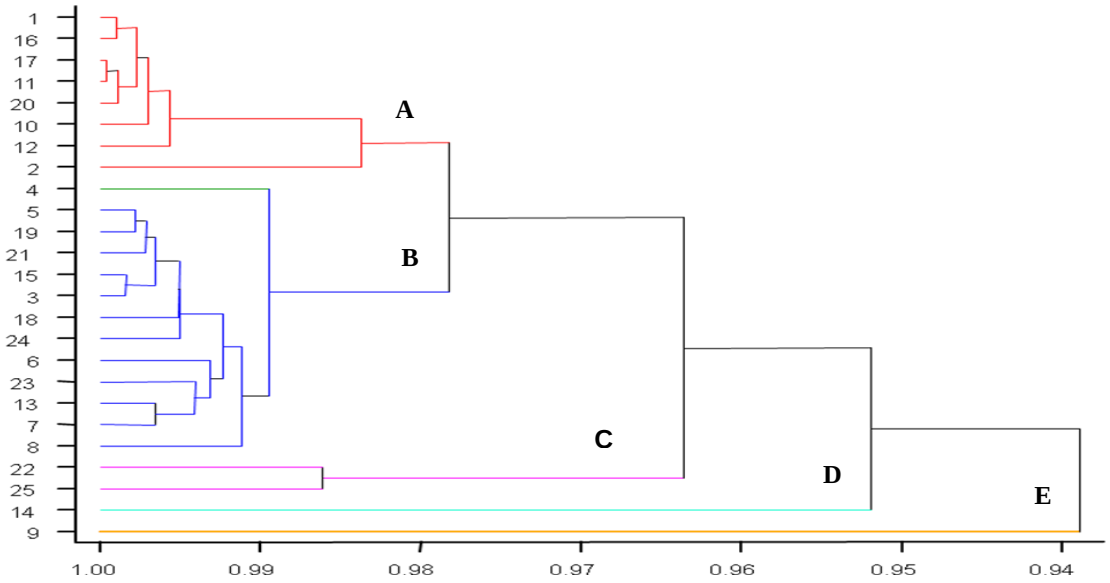

Figure 2. Dendrogram from UPGMA cluster analysis of genotypes based on drought tolerance indices GMP, MP, STI, YI, TOL, YSI and SSI) and grain yield of sorghum accessions, in both irrigated and drought stress condition (for genotype codes: see Table 1).

drought stress, but failed to select genotypes with good yield in both conditions. Similar results were reported on barley (*Hordeum vulgare*) Rizza et al. (2004), on wheat (*Triticum aestivum*) Sio-Se Mardeh et al. (2006), on durum wheat (*Triticum durum*) Talebi et al. (2009); Shiri et al. (2010), and on chickpea (*Cicer arietinum*) Talebi et al. (2011). The significant positive correlation found between SSI and TOL, indicated that these indices are able to select susceptible genotypes.

The biplot vectors for the indices MP, STI, and GMP remained between the Y_{ir} and Y_s vectors, indicating that these indices are very similar for drought selection. In the current study, MP, STI and GMP appeared to be the best indices for dividing the angle symmetrically between Y_{ir} and Y_s. Therefore, these factors can be used to select for genotypes that are better adapted to both conditions. Similar results were reported by Yarnia et al. (2011) on rapeseed (*Brassica napus*). Darvishzadeh et al. (2010) examined sunflower (*Helianthus annuus*) in one location, and found that tolerant indices including MP, STI and GMP were suitable for drought-tolerant genotype selection. However, based on the biplot presented by these authors, GMP is the most appropriate index for selection under stressed and non-stressed conditions. Kharrazi and Rad (2011) suggested that MP and STI are useful indicators for selecting tolerant genotypes. In the cluster analysis, the high yielding and drought tolerant genotypes (1 = EG 469; 2 = EG 849; 10 = EG 836; 11= EG 883; 12 = EG 885; 16 = EG 711; 17 = EG 783 and 20 = EG 481) were grouped in one cluster while the susceptible and low yielding genotypes (9 = EG 815; 14 = EG 1224; 22 = B-35 and 25 = EG 843) grouped in the bottom cluster indicating the efficiency of the drought indices for classifying genotypes under both stress and non-stress conditions.

Conclusion

Yield and yield-related traits under drought stress conditions were positively correlated to yield and yield-related traits under well irrigated conditions. The indices STI, GMP and MP were used to identify tolerant genotypes that produced high yield under both irrigated and drought stress conditions. The indices YSI and YI were used to identify resistant genotypes that are stable in different conditions and produce high grain under stressed conditions. Based on these different methods of selection indices, the current study identified seven outstanding genotypes (EG 885, EG 469, EG 481, EG 849, Hamelmalo, EG 836 and EG 711) for post-flowering drought tolerance that can be used by breeders in sorghum improvement program and conservation of these landraces is important.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

Assessment of genetic variability and yield stability in chickpea (*Cicer arietinum* L.) cultivars in River Nile State, Sudan

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Eight chickpea cultivars (Shendi, Jabel Marra, Wad Hamid, Atmor, Hwata, Burgeig, Salwa and Matama) were evaluated for genetic variability, yield stability and contribution of yield attributes to seed yield. Field experiments were carried out for four seasons (2007/2008, 2009/2010, 2010/2011 and 2011/2012) at Hudeiba Research Farm in River Nile State, Sudan. Randomized complete block design with six replications was used. Most of the studied traits recorded highly significant difference (P≤ 0.01) due to cultivars, seasons and their interaction. High heritability and low level of differences among phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for studied traits indicated that cultivars influenced more in the expression of these traits. Based on the stability analysis for seed yield; the top yielding cultivars Burgeig and Hwata were adapted to favorable conditions. Both cultivars were late in flowering and maturity and had high number of seeds plant^{-1}, biomass and harvest index. The cultivar Atmor with an intermediate seed yield was the most stable cultivar across seasons. The cultivar Salwa is optional due to its relatively high yield and large seed size. Combining farmer-preferred traits such as high and stable yield, large seed size, plant type and maturity into new cultivars will remain the main objective of the chickpea breeding program in Sudan.

Key words: Chickpea cultivars, genetic variability, yield stability, Sudan.

INTRODUCTION

Chickpea (*Cicer arietinum*) is an important pulse crop in the world as a source of diet for human and livestock and ranks third after dry bean and dry pea. Chickpea could fit well into rotation with cereal crops to improve soil fertility, prevent the build-up of diseases, insects and weeds. Chickpea is currently grown on about 12 million hectares worldwide with average annual production of 10.9 million tons (FAO, 2010). About 95% of chickpea cultivation and consumption is in the developing countries (Kassie et al., 2009).

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The seed yield of chickpea is influenced by many factors including genotype, growing season, geographical site, and agronomic practices (Tawaha et al., 2005). Even within an environment, seasonal climatic fluctuation requires the development of cultivars with consistence performance across environments to minimize the risk of failure in unfavorable seasons.

In Sudan chickpea is traditionally grown as a winter crop in the northern part, however, its production has expanded recently to the central clay plain of central Sudan. The growing season is restricted to a short period of time by the high temperatures prevailing at the beginning and end of season. The chickpea yields in Sudan vary from 0.83 to 2.8 t/ha, depending on weather conditions (Ahmed et al., 1995). Phenotypically stable genotypes are of great importance where seasonal fluctuations are large. Although a number of cultivars have been recommended for the cultivation, information on genotypic variability, yield stability and the contribution of yield-related traits to the yield performance of chickpea cultivars are scare. Therefore, the objectives of this study were to determine the magnitude of genotypic variability for traits of interest, investigate the contribution of yield attributes in seed yield and assess cultivars yield stability and seasonal adaptation.

MATERIALS AND METHODS

Eight released chickpea cultivars (Shendi, Jabel Marra, Wad Hamid, Atmor, Hwata, Burgeig, Salwa and Matama) were evaluated for four seasons (2007/08, 2009/10, 2010/11 and 2011/12) at Hudeiba Research Farm (HRF), Ed-dammer (17° 34` N, 33° 56` E, and 350 m above sea level), located in the River Nile State, Sudan. The pedigree and a brief description of the eight cultivars are given in Table 1. Monthly data for temperature and relative humidity across seasons is illustrated in Table 2.

Sowing date was in mid-November in all seasons. Each cultivar was sown in two rows 6 m long, 0.6 m apart and 0.1 m between plants within the row. Irrigation was done every 10 days. A starter dose of nitrogen in the form of urea urea was applied at a rate of 20 kg N/ha with the third irrigation. The plots were hand weeded twice at early stages of crop cycle. The insecticide spinosad (Tracer 240) was used against African boll warm.

Data were collected on days to 50% flowering, days to 90% maturity, plant height (cm), seed yield (t ha^{-1}), biomass (t ha^{-1}) and harvest index (%). Average numbers per plant of filled pods, empty pods, seeds number, seed yield gram/plant and 100-seed weight were estimated from five randomly selected plants.

The cultivars were arranged in RCB design with 6 replicates. Separate analysis of variance for each season was performed for seed yield and its component before running the combined analysis. The mean differences were separated using Duncan's multiple range test (DMRT). The genetic parameters and broad-sense heritability were estimated as suggested by Burton (1952) and Hanson et al. (1956).

Each season was used as a separate environment to measure stability parameters following regression analysis. Both Wricke's (1962) ecovalence (Wi) and Eberhart and Russell (1966) models were employed to investigate yield stability. General analysis was done using a computer program of GenStat 12th edition.

RESULTS AND DISCUSSION\

Genotypic variability

The tested cultivars differed significantly in all studied traits indicating their genetic variability as shown from their diverse origin. Separate analysis of variance for seed yield (t ha^{-1}) showed highly significant differences among cultivars in each season. Maximum, minimum, range, standard error, error mean square and coefficient of variation for seed yield in each season are given in Table 3. Mean seed yield varied among environments (seasons) and ranged from 1.79 t ha^{-1} in season 2010/11 to 2.38 t ha^{-1} in season 2009/10.

Mean squares of 11 traits of the eight chickpea cultivars in the four environments (seasons) are shown in Table 4. There were significant differences among cultivars, seasons and their interaction ($P \leq 0.01$) for most of the studied traits. Non-significant difference between cultivars for number of filled pods and seed yield/plant (g) was due to low genetic effect and large environmental effect. Furthermore, the non-significant difference of season X cultivar interaction for some traits indicated that the performance of the cultivars with respect to these traits was consistent across seasons.

Table 5 shows the means values of some important traits of eight chickpea cultivars across four seasons. Days to 50% flowering of the eight cultivars ranged from 38 to 48 days whereas days to 90% maturity ranged from 98 to 109 days. The cultivar Matama was the earliest in flowering and maturity, whereas Hwata was the latest in flowering and Atmor was the latest in maturity. Similar results were reported in several earlier studies that showed significant variation in days to flowering and maturity in chickpea (Atta et al., 2008; Saleem et al., 2008). Early maturity when combined with high seed yield is a desirable trait that could help to avoid terminal heat and drought and increase its adaptation in the sub-tropics (Kumar and Rao, 2001; Upadhyaya et al., 2007).

Plant height is a desirable trait to reduce lodging and enhance mechanical harvest in crops. Significant difference was observed in plant height of the eight cultivars and ranged from 47 cm for Matama to 54 cm for Atmor and Jabel Marra (Table 5). The range in plant height of the eight cultivars is narrower than the range of 30 to 70 cm reported by Gaur et al. (2010). This might be due to the selection pressure imposed in these cultivars for the desirable height for chickpea production in Sudan.

Higher number of pods, seeds and seed weight contribute to higher seed yield. Significant differences

Table 1. Cultivar pedigree and some descriptive characters.

Cultivar name	Accession No.	Year of release	Genetic background (pedigree)	Growth habit	Wilt/ root rot disease
Shendi	ILC 1335	1987	Afghanistan Selection	Semi-spreading	Susceptible
Jabel Marra	ILC 915	1993	Iran(Vysokoroshyj 30) Selection	Semi-erect	Susceptible
Wad Hamid	ICCV 2	1996	India-ICRISAT Selection	Spreading	Resistant
Atmor	ICCV 89509	1996	(L 550/Radhey)//(K 850/H 208)	Semi-erect	Resistant
Hwata	ICCV 92318	1998	(ICCV2/Surutato 77)//ICC 7344	Semi-erect	H. Resistant
Burgeig	ICCV 91302	1998	ICCC32/(K4/Chafa)	Semi-erect	H. Resistant
Salwa	FLIP 89-82c	1996	(X87TH 186/ ICCI 4198)//FLIP 82-150C	Spreading	Resistant
Matama	FLIP 91-77c	1998	(X89TH7/ILC 1245)//FLIP 82-150C	Semi-Spreading	Susceptible

Table 2. Monthly maximum, minimum temperature and relative humidity at Hudeiba Research Farm during 2007/08, 2008/2009, 2009/2010 and 201020/11 seasons.

Month	Maximum temperature (°C)				Minimum temperature (°C)				Mean relative humidity (%)			
	2007-2008	2009-2010	2010-2011	2011-2012	2007-2008	2009-2010	2010-2011	2011-2012	2007-2008	2009-2010	2010-2011	2011-2012
November	34.3	32.8	35.4	30.4	20.6	18.4	22.1	16.3	50	40	53	37
December	31.8	31.0	31.7	31.2	17.5	14.7	17.1	16.3	53	43	52	50
January	28.5	32.0	28.9	28.6	21.5	16.1	13.0	12.5	51	50	45	41
February	31.0	33.6	33.4	33.5	14.8	16.1	16.1	17.1	47	43	36	53
March	38.7	36.4	34.5	34.3	19.2	19.5	18.2	17.5	30	30	34	31

Table 3. The mean, maximum, minimum, range, error mean square, standard error and coefficient of variation for seed yield (t ha^{-1}) for four environments (seasons).

Season	Mean	Maximum	Minimum	Range	EMS	5%LSD	SE±	CV%
2007/08	1.82	1.99	1.72	0.27	0.084**	0.3397	0.118	15.9
2009/10	2.38	2.74	1.93	0.81	0.146**	0.3397	0.156	16
2010/11	1.79	2.02	1.46	0.56	0.192**	0.3397	0.179	24.5
2011/12	1.92	2.47	1.71	0.76	0.072**	0.3397	0.11	14

were found among cultivars in number of seeds/plant, number of empty pods and 100-seed weight (Table 5). The cultivar Jabel Marra attained the highest number of seeds per plant but the lowest 100-seed weight. Conversely, the two cultivars Matama and Salwa were recorded the lowest number of seeds per plant but had the highest seed weight. This explained the strong negative correlation between the two traits ($r = -0.876$, $P < 0.01$). The empty pods ranged from 14 pods per plant for Hwata to 7 pods per plant for Matama. Generally, the high yielding cultivars showed high number of empty pods.

Cultivars differed significantly in biomass production (Table 5) as reported by other investigators (Arshad et al., 2004; Jeena et al., 2005). The three top yielding cultivars (Hwata, Burgeig and Jabel Marra) gave the highest biomass indicating the high contribution of this trait to seed yield of these cultivars. Significant difference was found among cultivars in harvest index (Table 5). The highest harvest index was recorded by Matama cultivar indicating its efficiency in translocation of assimilates for seed yield despite its earliness. The significant correlation with seed yield ($r = 0.794$, $P = 0.016$) suggested the importance of harvest index as a key selection trait as reported earlier (Krishnamurthy et al., 2011) especially under heat stress conditions.

Genetic parameters for yield and its components are given in Table 6. Phenotypic coefficient of variability

Table 4. Mean squares of yield and some yield components for eight chickpea cultivars evaluated for four seasons (2007/2008, 2009/2010, 2010/2011and 2011/2012).

Trait	Season(df = 3)	Genotype(df = 7)	Seas.xGen.(df = 21)	Pooled error(df = 140)
Days to 50% flowering	138**	375**	50.1**	8.99
Days to 90% maturity	863**	239**	72.2**	22.2
Plant height	4896**	150**	26.0ns	21.2
Number of full pods	2675**	539ns	272ns	273
Number of empty pods	1080**	139**	47.9**	19.3
Number of seeds/plant	2847**	1806**	376ns	352
100-seed weight (g)	42.2**	545**	5.76**	2.07
Seed yield/plant(g)	93.8**	19.7ns	17.0ns	11.5
Biomass (t ha^{-1})	3.35ns	1.48**	2.01**	0.61
Harvest index (%)	0.09**	0.02**	0.007**	0.003
Seed yield (t ha^{-1})	3.68**	0.89**	0.41**	0.123

Table 5. Means of some vegetative and reproductive traits for eight chickpea cultivars evaluated for four seasons (2007/2008, 2009/2010, 2010/2011and 2011/2012).

Cultivar	Days to 50% flowering	Days to 90% maturity	Plant height (cm)	Biomass (t ha^{-1})	Harvest index (%)	Filled pods number	Empty pods number	No. of seeds /plant	100-Seed weight	Seed yield g/plant
Shendi	46^{b}	104^{c}	51.6^{ab}	5.28^{bc}	0.339^{cd}	49^{ab}	12^{ab}	59^{ab}	19.4^{e}	10.4^{ab}
Jabel Marra	42^{c}	104^{bc}	53.8^{a}	5.66^{ab}	0.358^{bc}	51^{a}	10^{bcd}	65^{a}	17.3^{f}	10.7^{ab}
Wad Hamid	38^{d}	107^{ab}	49.9^{bc}	5.38^{bc}	0.320^{d}	44^{ab}	8^{de}	48^{bc}	24.7^{c}	10.0^{b}
Atmor	42^{c}	109^{a}	53.8^{a}	5.34^{bc}	0.360^{bc}	48^{ab}	9^{cde}	61^{a}	18.8^{e}	10.8^{ab}
Hwata	48^{a}	107^{abc}	50.6^{bc}	5.57^{abc}	0.396^{a}	52^{a}	14^{a}	61^{a}	22.5^{d}	12.5^{a}
Burgeig	47^{b}	107^{ab}	51.9^{ab}	5.90^{a}	0.380^{ab}	53^{a}	12^{abc}	56^{ab}	23.9^{c}	12.1^{ab}
Salwa	46^{b}	104^{c}	48.6^{cd}	5.43^{abc}	0.361^{bc}	44^{ab}	12^{ab}	44^{c}	31.9^{a}	12.0^{ab}
Matama	38^{d}	98^{d}	46.6^{d}	5.10^{c}	0.397^{a}	40^{b}	7^{e}	42^{c}	26.4^{b}	10.8^{ab}
Mean	44	105	50.9	5.46	0.364	48	10.5	55	23.1	11.2
SE±	0.61**	0.96**	0.94**	0.16*	0.011**	3.37NS	0.896**	3.83**	0.29**	0.69^{NS}
5%LSD	1.71	2.69	2.63	0.45	0.031	9.43	2.51	10.7	0.82	1.94
CV%	6.9	4.49	9.05	14.4	14.3	34.8	41.8	34.3	6.2	30.4

*, ** Significant at 0.05 and 0.01 levels of probability, respectively; NS= non-significant. Means followed by the same letter (s) within each column are not significantly different at 0.05 according to DMR

was slightly higher than genotypic one for all traits. High heritability estimates were recorded for all studied traits except biomass. These high estimates of heritability for the traits under consideration indicated that a reasonable proportion of the total variability was due to genetic causes. Khan et al. (2011) and Saleem et al. (2008) found similar results and observed high heritability values in chickpea for days to flowering, plant height and 100-seed weight. Estimates of genetic advance suggested that number of seeds $plant^{-1}$, 100-seed weight, and days to 50% flowering were important traits to select for high yield.

The potential of the crop to respond favorably to breeding programs depends upon the nature and magnitude of the variability. The yield potential of the two cultivars Burgeig and Hwata can be explained based on the high values for full pods number, number of seeds per plant, biomass, tall height and late maturity. The two cultivars were land races introduced from ICRISAT. The two cultivars Salwa and Matama recorded considerable seed yield, high large seed, early flowering and maturating and had the shortest plant height. Both cultivars were land races introduced from ICARDA. Depending to these different traits and due to their origin the cultivars could be cluster in two diverse groups.

Table 6. Broad Sense Heritability (h^2BS); genetic advance (GA %); genotypic coefficient of variation (GCV%); phenotypic coefficient of variation (PCV%) and coefficient of variation (CV%) of yield and some yield components for eight chickpea cultivars evaluated for four seasons.

Trait	GCV	PCV	h^2BS	GA%	CV%
Days to 50% flowering	23.8	25.5	87.1	19.9	6.9
Days to 90% maturity	7.8	9.4	68.5	13.9	4.5
Plant height	12.8	15.8	66.0	10.9	9.1
Number of empty pods	59.1	75.4	61.5	10	41.8
Number of seeds/plant	41.3	53.8	58.9	35.7	34.3
100-seed weight (g)	54.5	55	98.3	25.7	6.2
Biomass (t ha^{-1})	10.8	20	29.2	0.66	14.4
Harvest index (%)	18.9	25.1	56.2	0.11	14.3
Seed yield (t ha^{-1})	25	32.6	58.7	0.78	17.8

Table 7. Analysis of variance for stability for seed yield (t ha^{-1}) of eight chickpea cultivars evaluated across four environments.

Source of variation	DF	SS	MS	F	Percent explained
Genotypes (G)	7	6.25	0.893	7.23**	24.1
Environment (E)	3	11	3.68	7.53**	42.54
GE interaction	21	8.66	0.412	3.34**	33.4
Environment (Linear)	1	11	11	22.6**	
GE interaction (Linear)	7	4.21	0.601	4.87**	
Pooled deviation	14	4.45	0.318	2.57**	
Pooled error	140	17.3	0.124		
Total	191	53			

**Significant at 0.01 levels of probability.

Yield satiability

Analysis of variance for stability showed highly significant differences for seed yield among genotypes (G), environments (E) and their interactions (GEI) (Table 7). From the total sum of squares due to treatments (G + E + GEI), environment attributed the highest proportion of the variation (42.5%), followed by genotype x environment interaction (33.4%), whereas genotype contributed 24.1% of total variation. The sum squares due to environments and genotype x environment were partitioned into environments (linear), genotype x environment (linear) and deviations from the regression model. The significance of these components showed that both predictable and unpredictable (seasons) components shared G x E interaction. The G x E (Linear) interaction was highly significant which demonstrated that genotypes responded differently to various environmental conditions in agreement with that reported earlier in chickpea (Arshad et al., 2003; Bakhsh et al., 2006; Prakash, 2006).

The mean seed yield of the eight chickpea cultivars ranged from 2.3 t ha^{-1} for Burgeig to 1.7 t ha^{-1} for Wad Hamid. According to Eberhart and Russell (1966), both the linear (bi) and non-linear (s^2_{di}) are needed for judging the stability of a genotype. The stable genotype would be the one with high mean yield, low regression coefficient (bi=1) near unity, with non-significant deviation from regression ($s^2d=0$). Regression values above unity (>1) describe genotypes specifically adapted to high yielding conditions whereas genotypes with slope less than one (<1) are sensitive to change in environment and are therefore, better adapted to poor environments (Finlay and Wilkson, 1963). Moreover, Wricke (1962) reported that the low values of Wi are indicative of high stability. The regression coefficients (bi values) ranged from 0.05 to 1.85 for seed yield (Table 8). This large variation indicated the differential responses of cultivars to seasonal variations. The two cultivars Mattama and Atmor recorded high seed yield, regression coefficient close to unity with non-significant deviation from regression coefficient and low values of

Table 8. Mean yield, regression coefficient (bi), deviation from regression (s^2_{di}) and ecovalance (Wi) on seed yield (t ha^{-1}) of eight chickpea cultivars evaluated across four environments.

Cultivar	Mean	bi	s^2_{di}	Wi
Shendi	1.770^{cd}	0.05	0.038	0.284
Jabel Marra	1.999^{b}	0.27	0.139	0.401
Wad Hamid	1.709^{d}	1.51	0.05	0.16
Atmor	1.922^{bc}	0.66	0.035	0.096
Hwata	2.224^{a}	1.85	0.04	0.247
Burgeig	2.261^{a}	1.45	0.045	0.135
Salwa	1.970^{bc}	1.48	0.017	0.087
Matama	1.950^{bc}	0.73	0.008	0.034
Mean	1.976			

Means followed by the same letter (s) within each column are not significantly different at 0.05 according to DMRT.

ecovalance (Wi) indicating their stability across seasons.

Salwa cultivar showed similar results, however, it had a regression values above unity (bi= 1.5) indicating its adaptation to high yielding conditions. The two cultivars with the highest seed yield, Burgeig and Hwata showed regression (bi) value more than unity and non-significant deviation from regression indicating their specific adaptation to favorable environments. On the other hand, Jabel Marra had high seed yield but regression coefficient less than unity and non-significant s^2_{di} values indicating that the cultivar could be considered as adapted to unfavorable conditions.

Conclusion

The high genotypic variation observed in most of the studied traits coupled with high broad sense heritability estimates indicated the genetic influence and hence the possibility of genetic improvement in the traits under consideration. The cultivars used in this study showed different levels of stability across different environments. Atmor had stable yield across seasons, therefore it could be tested over locations for stability verification and for further use in breeding program. The two cultivars Burgeig and Hawata were adapted for favorable conditions and are recommended for farmers in the favorable production areas. Salwa could be a farmer-preferred cultivar due to its relatively high seed yield and large seed size. Performance of high yielding cultivars associated with high harvest index, this suggests the importance of harvest index as key selection traits.

Conflict of Interest

The authors have not declared any conflict of interest.

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Differential morphological, physiological, and molecular responses to water deficit stress in sugarcane

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This study was designed to characterize morphological, physiological and molecular responses of sugarcane genotypes to a simulated water deficit stress. Two genotypes (TSP05-4: Drought-tolerant; TCP02-4589: Drought-sensitive) were subjected to a 20-day water deficit treatment and an 8-day recovery period. Leaf photosynthesis (P_n), transpiration rate (E), stomatal conductance (G_s), leaf greenness index (SPAD) and variable-to-maximum chlorophyll *a* fluorescence ratio (F_v/F_m), were evaluated before, during and after water deficit. Root-to-shoot ratio (R/S), stalk height (SH), diameter (SD) and stalk weight (SW) were evaluated at the end of the experiment. Real-time RT-PCR confirmed seven differentially-expressed transcript-derived fragments (TDFs) identified by cDNA-AFLP. P_n rates were similar between the genotypes under well-watered conditions. However, under water deficit, TSP05-4 had higher P_n rates. SPAD, F_v/F_m and R/S were also generally higher in TSP05-4, regardless of soil moisture status. Water deficit-induced reductions in SH and SW were greater in TCP02-4589 than in TSP05-4. Three TDFs showing sequence similarities to genes encoding a putative expressed pentatricopeptide, a protein kinase CK2 regulatory subunit CK2β3, and a glucose-6-phosphate/phosphate translocator 2 were identified in TCP02-4589. One TDF similar to a drought-inducible protein was identified in TSP05-4. Recovery of physiological processes and gene expression patterns to the water stress levels was fast.

Key words: Differential gene expression, water deficit stress, re-watering, *Saccharum* spp.

INTRODUCTION

Sugarcane (*Saccharum* sp.) is an economically important crop that is cultivated in more than 90 countries for sugar, ethanol and biomass production. Since sugarcane production is concentrated in many regions where water supply is either inadequate or irrigation infrastructures are underdeveloped, water deficit stress is a major limitation

to optimal productivity of this crop (Inman-Bamber and Smith, 2005). Developing varieties that use water more efficiently is, therefore, an important goal for sugarcane improvement programs.

The period between 60 and 150 days of crop age, known as the formative phase, has been shown to be very sensitive to water deficit stress in sugarcane (Naidu, 1976). Water deficit during this phase has been shown to adversely affect gene and protein expression, morphological, physiological and biochemical traits, and consequently, cane and sugar yields (Rocha et al., 2007; Silva et al., 2008; Cha-um and Kirdmanee, 2009; Rodrigues et al., 2009).

Plasticity in adjusting to and recovering from drought is often overlooked in drought response studies, even though these mechanisms can enhance crop survival during water shortages (Ashton, 1956; Inman-Bamber, 1995). A better understanding of these responses and mechanisms is necessary in developing guidelines and procedures to efficiently screen germplasm for stress tolerance.

Studies have focused on understanding the morphological, physiological and molecular processes responsible for high performance under drought conditions in sugarcane (Inman-Bamber and Smith, 2005; Rocha et al., 2007; Silva et al., 2008; Cha-Um and Kirdmanee, 2009; Rodrigues et al., 2009; Zingaretti et al., 2014). However, few studies have integrated these processes to achieve progress in genetic improvement of sugarcane drought tolerance. Such studies may contribute to the development of tolerant genotypes, either for transgenic plant development or for marker-assisted breeding.

The objective of this study was to characterize morphological, physiological and molecular responses to water deficit stress and to re-watering in two sugarcane genotypes.

MATERIALS AND METHODS

Plant materials and growth conditions

This study was conducted from March to July, 2009, in a greenhouse at the Agrilife Research and Extension Center, Weslaco, Texas, USA. Two sugarcane genotypes classified as either drought tolerant (TSP05-4) or sensitive (TCP02-4589) were used. Two-week plantlets, transplanted into 15-L pots containing MM200 substrate, were watered daily, and fertilized two times per week with 10N-4.4P-8.3K (Peter's Corp., St. Louis, Mo.) until 69 days after planting (DAP). The average daily photosynthetic photon flux at canopy level was 15±3.8 $mol \cdot m^{-2}$. Average day/night temperatures were 28.8±4.4 / 21.7±3.2°C and average day/night relative humidity values were 48±11/68±11%. At 70 DAP, irrigation treatments were initiated. Plants of each genotype were randomly divided into one group subjected to a water deficit stress regime by maintaining volumetric soil moisture (VSM) at ~15%, and another group being well-watered (VSM ~ 35%). Volumetric soil moisture contents were monitored continuously using soil moisture sensors (EC5, Decagon Devices, Inc) connected to data loggers (Em5b, Decagon Devices, Inc). Irrigation treatments were maintained until 90 DAP.

Physiological and morphological analyses

Leaf photosynthesis (P_n), stomatal conductance (G_s), transpiration rate (E), variable-to-maximum chlorophyll *a* fluorescence ratio (F_v/F_m), leaf greenness index (SPAD) and leaf relative water content (RWC) were evaluated at two days before water deficit stress initiation (T_0), at two, twelve and twenty days after initiation of water deficit stress treatment (T1, T2 and T3, respectively), and at eight days after re-watering (T4).

The traits P_n, G_s and E were measured using a portable gas exchange system CIRAS-2 (PPSystems) under ambient temperature, light saturation (1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and CO_2 partial pressure of 35 Pa. A pulse amplitude modulation fluorometer (Model OS5-FL, Opti-Sciences, Tyngsboro, MA, USA) was used to measure F_v/F_m of leaves dark-adapted for 30 min. F_v is the variable fluorescence (F_m-F_0), Fm is the maximal fluorescence yield following a saturating pulse of light and F_0 is the minimal fluorescence yield in the absence of actinic light.

Leaf greenness index (SPAD) measurements were made with a Minolta SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, NJ, USA). The measurements of SPAD, P_n, G_s and E were completed between 10:00 and 12:00, using the 3rd leaf from the top-most visible dewlap of stalk. Following gas exchange and SPAD measurements, leaf disks (1 cm^2) were sampled from each plant and used for RWC determination. Leaf tissue samples for RWC determination were collected around 15:00. RWC was calculated following the method of Matin et al. (1989).

Following physiological measurements at T4, plants were harvested and root-to-shoot ratio (R/S), stalk height (SH), stalk diameter (SD) and stalk weight (SW) were measured. Plants were harvested at soil level and divided into leaves, stems and roots. SH was measured from the base of the top-most visible dewlap to the soil level while SD was measured with a pair of calipers at 10 cm from the soil level. Individual biomass components were oven-dried (70°C, 72 h) and the dry weight data were used to calculate R/S.

cDNA-AFLP analysis

Leaf tissue samples were collected at the T1, T2 and T4 evaluation times from three plants of each treatment. Tissue samples were immediately frozen in liquid nitrogen and stored at -80ºC. Total RNA extraction and Poli $(A)^+$ RNA isolation were achieved using the QIAGEN Rneasy Plant Mini Kit (Qiagen, Valencia, CA) and MicroPoly(A) Purist™ Kit (Ambion), respectively, following the manufacturer's instructions. Single and double stranded-cDNAs were synthetised using the SuperScript™ Double-Stranded cDNA Synthesis (Invitrogen).

cDNA-AFLP analysis was performed using the AFLP® Expression Analysis Kit of LI-COR (LI-COR, Lincoln, NE). Restriction enzymes *Taq*I and *Mse*I were used to digest the cDNA and to generate pre-amplification PCR products. Selective PCRs were performed with 22 primer combinations obtained by the eight *Mse*I+2 primers and the eight *Taq*I+2 primers, where +2 represents two selective nucleotides: +GA, +GT, +TC, +TG, +CT, +CA, +AG and +AC on both adaptor primers. The *Taq*I+2 selective primers were labeled with 700 and 800-nm infrared dye (LI-COR, IRDye 700 and IRDye 800). Selective PCR products were resolved on 6.5% denaturing polyacrylamide gels in a LI-COR DNA analyser (model 4300 LI-COR®). Eletrophoretic run parameters were: 1500 V, 40 W, 40 mA, 45°C, 25-min pre-run and 2-h main run. Data images were collected using LI-COR's Saga AFLP Analysis Software.

Isolation of differentially-expressed TDFs was perfomed according to the AFLP® Expression Analysis Kit, mentioned previously.

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Table 1. Primer sequences used for real-time RT-PCR analysis.

Primer ID	bp	Forward primer (5'-3')	Reverse primer (5'-3')
JG014679	120	CCCTCAAATGCAGGGAACTA	GCCAGCTGTTTTCTGAGACC
JG014680	84	CCTACGATGACGAGGTCCAT	CCTTTGCTGCAACAATTTCA
JG014675	65	AGCAACTAACCAACCCATCG	CTTGTTGGAGGGAGATCGAG
JG014677	79	AACGCCGAAACTTCTTCTGA	GAGTCGAACTCGGGAACTGA
JG014684	128	ATCTGGCAGGCGTGAGTTTA	TTCCACTGCTCACTTGCATC
JG014686	65	TTCTCCAAGAAGGGGATGAA	ATGGAGAGGCAGGCGTAGTA
JG014687	72	GCAGCAACCGGATATCTCTT	CTGCCTTGGCCTATTTCTTG

Bp, Amplicon size.

The TDFs excised from gels were purified using the Zymoclean™ Gel DNA recovery Kit (Zymo Research). The purified TDFs were subsequently cloned into the pGEM®-T Easy vector (Promega Corp., Madison, WI) and then used to transform *Escherichia coli* DH5α competent cells. Recombinant plasmids were isolated using Zyppy™ plasmid Miniprep kit (Zymo Research). Purified plasmids containing the insert were sequenced using an automated DNA sequencer (Applied Biosystem, Inc.) at Iowa State University's, DNA Facility (Ames, Iowa, USA).

Nucleotide and translated sequences were analysed for homology with nucleotide and protein sequences available in the GenBank (http.//www.ncbi.nlm.nih.gov/BLAST) database using the BLASTx and BLASTn search tools, respectively.

Real-time RT-PCR analysis

Real-time reverse transcription PCR (RT-PCR) was used to confirm 7 TDFs isolated. The primers (Table 1) were designed using the Primer 3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and compared to the NCBI database using the Blast tool to verify the specificity of sequences. RT-PCR reactions were performed using iQ™ SYBR® Green Supermix (Bio-Rad laboratories, Hercules, CA, USA). The glyceraldehide-3-phosphate de-hydrogenase (*GAPDH*) gene was used as an endogenous reference gene (Iskandar et al., 2004). The reactions were performed for each sample in triplicates. The following amplification program was followed using a BioRad iCycler iQ5 thermocycler (Bio-Rad laboratories, Hercules, CA, USA): 50ºC for 2 min, 95ºC for 10 min followed by 40 cycles of 95ºC for 15 s and 60ºC for 1 min. The level of relative gene expression of each fragment normalized to the endogenous reference gene were calculated using the $2^{-\Delta(\Delta Ct)}$ method (Livak and Schmittgen, 2001).

Experimental design and statistical analysis

The physiological experiment setup was a split-plot in time arrangement, with evaluation times as the main plot, and water supply regimes and genotypes as sub-plots replicated four times. The growth experiment setup was a split-plot, with water supply as the main plot, and genotypes as sub-plots replicated four times. Analysis of variance was performed at probability of 5%.

RESULTS AND DISCUSSION

Physiological and morphological analyses

Gas exchange parameters (P_n, E and G_s) were significantly reduced by water deficit stress in both genotypes (Figure 1). Prior to initiation of water deficit stress (T0), there were no significant differences in these parameters between the genotypes. By the evaluation times T1, T2 and T3, however, stress-induced differences were observed.

Under well-watered conditions, the average gas exchange parameters of both genotypes were similar (Figure 1). Under water deficit, however, the average P_n, E and G_s of TSP05-4 were, respectively, about 48, 31 and 33% higher than those of TCP02-4589. The lower reduction in G_s of TSP05-4 may explain why its P_n values were also higher than those of TCP02-4589 under water deficit stress. Regulation of water loss through transpiration by stomata is a well-known mechanism for maintaining higher and favorable plant water status, which in turn allows the plant to sustain physiological processes under mild water deficit conditions.

Despite the higher reductions in gas exchange parameters of TCP02-4589, RWC did not differ between the tolerant and sensitive genotypes (Figure 1). RWC values of TCP02-4589 were still high (above 80%) compared to results found in other sugarcane studies (Jangpromma et al., 2007; Cia et al., 2012; Boaretto et al., 2014), which RWC values of sensitive genotypes were lower than 70% under severe water stress. A possible explanation for the lack of differences among drought-tolerant and sensitive genotypes is that water stress was not severe enough to influence the RWC.

A complete recovery of P_n, G_s, E, and RWC was observed for both genotypes when plants were re-watered for 8 days (T4), following the water deficit stress treatments (Figure 1). These results suggest a high degree of physiological plasticity of the sugarcane genotypes in response to changing water conditions, as has been reported for other sugarcane genotypes (Ashton, 1956; Inman-Bamber, 1995).

SPAD values differed between genotypes with TSP05-4 having higher values (~51) than TCP02-4589 (~43; Figure 1). Also, decline in F_v/F_m was slightly more severe in genotype TCP02-4589. The higher values of SPAD and F_v/F_m found in genotype TSP05-4 suggest a greater capacity for radiation capture and radiation use efficiency in this genotype.

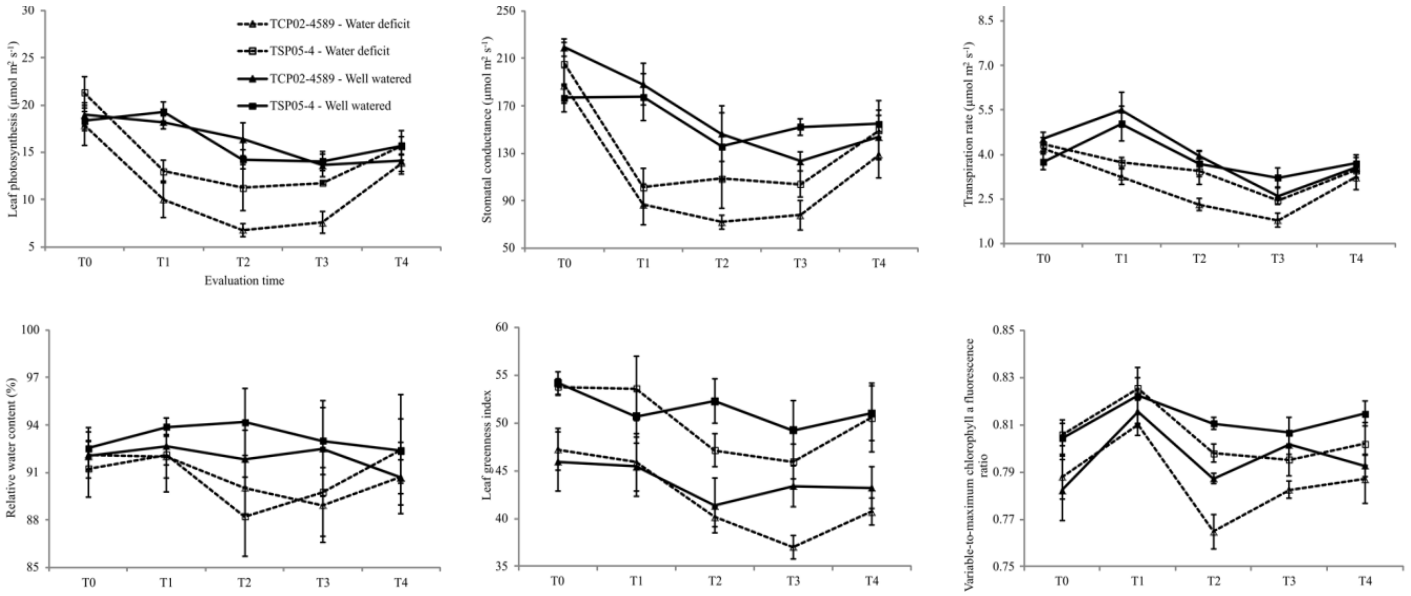

Figure 1. Effects of water deficit stress on leaf photosynthesis (P_n), stomatal conductance (G_s), transpiration rate (E), relative water content (RWC), leaf greenness index (SPAD) and variable-to-maximum chlorophyll *a* fluorescence (F_v/F_m). T0, Two days before water deficit stress initiation; T1, T2 and T3, two, twelve and twenty days after initiation of water deficit stress respectively; T4, eight days after re-watering conditions. Each value represents the mean ± standard error.

R/S ratio was higher in genotype TSP05-4 (0.62) compared to TCP02-4589 (0.36), regardless of water supply conditions (Figure 2). Under well-watered conditions, the average SH and SW of TCP02-4589 were, respectively, about 37 and 34% greater than that of TSP05-4. Stress-induced reductions in SH and SW of TCP02-4589, however, were much greater (about 22 and 31%, respectively) than those of TSP05-4 (about 4 and 13%, respectively). This maintenance of TSP05-4 probably resulted from higher values of P_n in this genotype. Despite these results, stalks of TCP02-4589 were heavier (about 13%, respectively) than those of TSP05-4, suggesting potential tradeoffs in productivity for survival. The average SD was not different between genotypes.

cDNA-AFLP analysis

About 1550 transcript-derived fragments (TDFs) were detected and an average of 70 TDFs per primer combination was produced. At least 30 TDFs were classified as differentially-expressed, with 24 TDFs being down-regulated and six up-regulated in response to water deficit. Twenty-three and six TDFs were detected exclusively in TCP02-4589 and TSP05-4, respectively, while one TDF was simultaneously present in both genotypes. Nineteen TDFs detected exclusively in TCP02-4589 were down-regulated, whereas in TSP05-4, 4 TDFs were down-regulated and 2 were up-regulated. These results show that at the molecular level, water stress responses were detected earlier in genotype TCP02-4589.

A total of 11 and eight differentially-expressed TDFs were exclusively detected at T1 and at T2, respectively, while 11 TDFs were detected at both evaluation times. One TDF was up-regulated at T1 and five at T2, while 10 TDFs were down-regulated at T1 and three at T2. All TDFs detected at both evaluation times were down-regulated.

In both genotypes, TDFs regulated by water deficit stress resumed their processes and normal expression patterns after the re-watering period. The ability to resume normal molecular and physiological functions indicated that the magnitude and duration of water deficit stress did not impair the ability for recovery.

Thirteen differentially-expressed TDFs were sequenced and its characteristics are described in Table 2. Five TDFs (JG014679, JG014675, JG014682, JG014686 and JG014687) showed significant similarity to genes with known or putative function, three (JG014680, JG014676, JG014684) were similar to hypothetical proteins and five (JG014681, JG014683, JG014677, JG014685 and JG014678) did not show significant similarity to any nucleotide sequence or protein in the non-redundant database.

Seven TDFs were selected for RT-PCR analysis and are indicated with an asterisk in Table 2. RT-PCR confirmed the regulation of five TDFs (JG014679, JG014680, JG014675, JG014684 and JG014686) while two (JG014677 and JG014687) did not show changes in the relative levels of gene expression between control

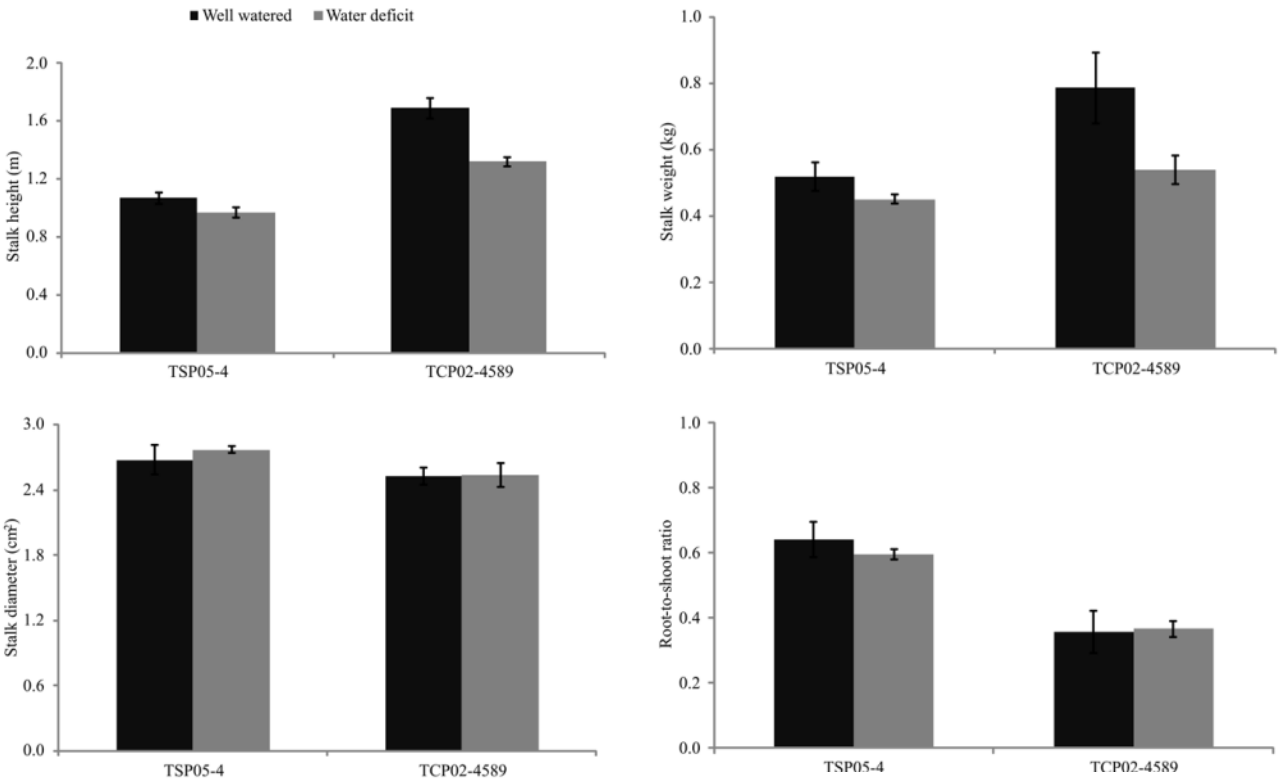

Figure 2. Effects of water deficit stress on stalk height, stalk weight, stalk diameter and, root-to-shoot ratio. Each value represents the mean ± standard error.

Table 2. Functional classification of differentially-expressed transcript derived fragments (TDFs) of two sugarcane genotypes (TSP05-4: T and TCP02-4589: S) regulated by water deficit conditions at two evaluation times: two and twelve days after initiation of water deficit stress treatment (T1 and T2, respectively).

Access GenBank [a]	TDF size	Sequence homology	*E*-value	Type [c]	G	ET
JG014679*	201	Putative expressed pentatricopeptide, *Oriza sativa* (ABA99065.2)	$1.0e^{-27}$	D	S	T1 and T2
JG014680*	247	Hypothetical protein OsJ-08616, *Oriza sativa* (EEE57919.1)	$2.0\ e^{-40}$	D	S	T1 and T2
JG014681	112	NSS	-	D	S	T1
JG014675*	84	22 kDA drought-inducible protein mRNA, *Saccharum* hybrid cultivar (AY496271.1)	$3.0\ e^{-33}$	U	T	T2
JG014676	93	Hypothetical protein OsI-08927, *Oriza sativa* (EEC74003.1)	$2.0\ e^{-07}$	D	T	T2
JG014682	264	Protein kinase CK2 regulatory subunit CK2β3, *Zea mays* (NM001111505.1)	$2.0\ e^{-79}$	U	S	T2
JG014683	217	NSS	$1.0\ e^{-19}$	U	S	T2
JG014677*	111	NSS	-	D	T	T1
JG014684*	143	Hypothetical protein LOC100273728, *Zea mays* (NP001141610.1)	1.0 e-^{19}	D	S	T1 and T2
JG014685	281	NSS	-	D	S	T1 and T2
JG014686*	282	Glucose-6-phosphate/phosphate translocator 2, *Zea mays* (NP001147439.1)	$1.0\ e^{-21}$	D	S	T2
JG014687*	343	Putative tocopherol polyprenyltransferase, *Oryza sativa* (BAC83059.1)	$5.0\ e^{-52}$	U	S	T2
JG014678	131	No significant similarity	-	D	S and T	T1

*TDFs selected for RT-PCR analysis; G, genotype; ET, evaluation time; [a] Access number of gene to NCBI database; [c]Classification of TDFs by expression patterns (D, down-regulated; U, up-regulated); nss, no significant similarity.

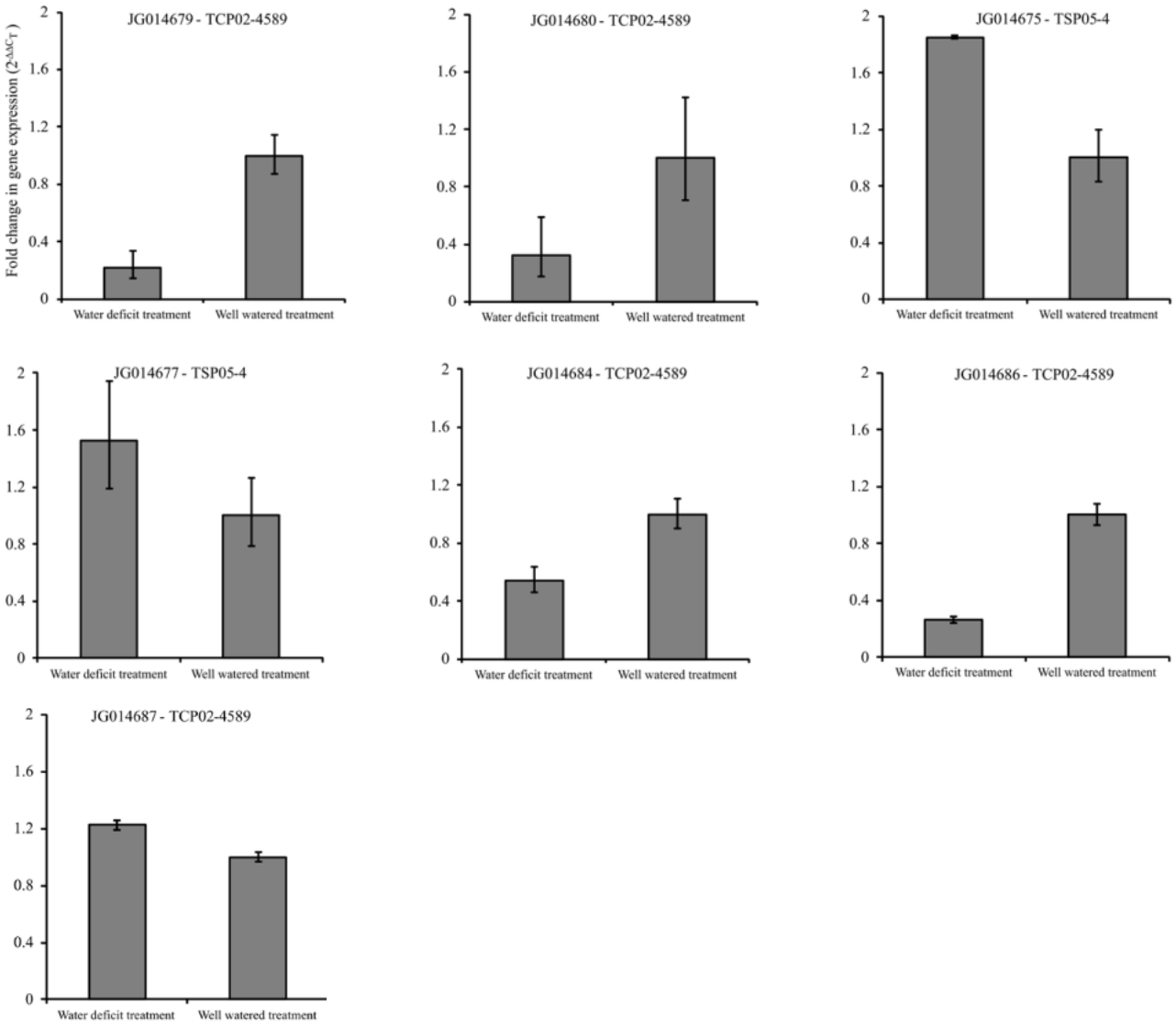

Figure 3. Fold change in expression of seven TDFs analyzed by RT-PCR in response to water deficit stress. All data were normalized to the glyceraldehide-3-phosphate de-hydrogenase (*GAPDH*) expression level.

and water deficit treatments (Figure 3).

Three TDFs showing significant sequence similarities to genes encoding a putative expressed pentatricopeptide (JG014679), a protein kinase CK2 regulatory subunit CK2β3 (JG014682) and a glucose-6-phosphate/phosphate translocator 2 (JG014686) were differentially-regulated in genotype TCP02-4589. JG014675 which is similar to a drought-inducible protein was differentially-regulated in TSP05-4 at T2. Also, one TDF (JG014687) similar to a tocopherol polyprenyltransferase gene was down-regulated in both genotypes at T2.

Pentatricopeptide repeat (PPR) is a protein family involved in plant development, organelle biogenesis, restoration of cytoplasmic male sterility, RNA processing and editing in mitochondria and chloroplasts, and responses to environmental stresses (Meierhoff et al., 2003; Lurin et al., 2004; Rodrigues et al., 2009). Under the water deficit conditions imposed in the present study, a PPR like-protein (JG014679) was suppressed at T1 and T2 in TCP02-4589. The suppression of JG014679 may have contributed to reduced P_n values observed in TCP02-4589 under water deficit. Two genes which show similarity to PPR proteins have also been previously observed in sugarcane genotypes exposed to waterdeficit stress (Rodrigues et al., 2009).

JG014686, which was also suppressed at T2 in TCP02-4589, is similar to a glucose-6-phosphate/phosphate translocator 2. Glucose-6-phosphate/phosphate translocator represents a distinct member of the phosphate translocator protein family and its proposed physiological functions include import of

glucose-6-phosphate into amyloplasts of heterotrophic tissues for use as a precursor for starch and fatty acid biosynthesis, and as a substrate for the oxidative pentose phosphate pathway (Fischer and Weber, 2002).

JG014682 showed similarity to a regulatory subunit CK2β3 of kinase CK2 and was induced at T2 in TCP02-4589. Several kinases have been reported to be regulated by drought conditions in sugarcane (Rocha et al., 2007; Rodrigues et al., 2009). Plant kinase CK2 protein, also known as casein kinase II, is involved in many different processes such as, DNA transcription, RNA translation and cell-cycle regulation (Riera et al., 2001; Espunya et al., 2005).

JG014675 was slightly induced at T2 in TSP05-4 and showed similarity to a drought-inducible protein mRNA (SoDip22) in *Saccharum officinarum* (Sugiharto et al., 2002). Because of the hydrophilic nature of SoDip22, and since the signaling pathway for its induction is, at least in part, mediated by ABA, it is plausible that it belongs to the abscisic acid, stress and ripening-induced (*Asr*) protein family and functions in drought adaptation.

In conclusion, leaf photosynthesis, leaf greenness index, variable-to-maximum chlorophyll *a* fluorescence ratio, root-to-shoot ratio, stalk height, and stalk weight of two sugarcane genotypes responded to water deficit stress in a manner that is consistent with their classification as drought tolerant or sensitive. Water deficit effects were detected earlier in the sensitive genotype TCP02-4589, since a higher number of differentially-expressed, transcript-derived fragments were detected in this genotype. The fast and complete recovery of physiological processes and gene expression patterns after re-watering demonstrate a high degree of physiological and molecular plasticity in response to changing water conditions.

Conflict of Interest

The authors have not declared any conflict of interest.

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Variability of root and physiological traits of different maturity groups of maize (*Zea mays* L.)

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Maize (*Zea mays* L.) is a major commercial crop, with high potential for production due to high solar radiation and low night temperature in sub-Saharan Africa. It is also the second most susceptible to drought among cereals, although phenotypic traits can be altered to improve drought resistance. Pot and field experiments were conducted to study the variability in root and physiological traits in different maturity groups of maize. Genotypes used were Sammaz 14, Sammaz 29, 2009 EVDT, 2009 TZE–W, TZE COMP-5 and 2009 TZEE, laid out in a Randomized Complete Block Design with 3 replications. The results obtained revealed no significant difference among the genotypes. However, the genotypes showed a good response to leaf temperature, canopy temperature, stomatal conductance and chlorophyll content. Variability was observed in three traits; days to anthesis, silking and anthesis silking interval. There was a significant correlation in leaf temperature in relation to fresh root weight, fresh shoot weight, dry shoot weight, dry root weight and shoot length. Root traits had positive relationship with grain yield. The genotypes had good rooting pattern development and combine with their physiological response they could be hybridized to develop drought tolerant varieties.

Key words: Correlation, drought stress, maize, maturity group, physiological traits, roots.

INTRODUCTION

Achieving food security; the first step towards poverty alleviation, is one of the biggest challenges facing developing countries. In most of Africa, food production is supplemented with imports to minimize the impact of shortages. Taking a cue from the most agriculturally advanced countries, it could be hypothesized that agriculture, hence food security in sub-Saharan Africa, will develop on a grain base. In West and Central Africa this crop is likely to be maize, which has evolved from a backyard crop to a major commercial crop providing food, animal feed and industrial raw materials (Badu-Apraku et al., 2009).

In general, average yields in tropical and sub-tropical regions are far lower than in temperate ones, with sub-Saharan Africa way below other regions with average values across countries of around 1 t ha^{-1}. This is in spite the fact that maize is one of the main crops in these regions, where the effects of climate change including rising temperatures, evapotranspiration losses and eventually, decreasing rainfall are expected to be particularly negative (World Bank, 2007). The possibilities for alleviation of water stress are limited. The majority

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of tropical maize is grown under rain-fed conditions and poor farmers from these regions are unable to implement crop management strategies that might at least mitigate some constraints (Araus et al., 2012).

Maize (*Zea mays* L.) has high potential for production and productivity in the savanna ecology of sub-Saharan Africa due to high solar radiation and low night temperatures. It is mostly grown under rain-fed conditions and among the cereals, it is the second most susceptible to drought next to rice. Annual maize yield loss due to drought is estimated to be 15% in West and Central Africa and losses may be higher in the marginal areas where the annual rainfall is below 500 mm and soils are sandy or shallow (Edmeades et al., 1995). Drought resistance might be increased by improving the ability of the crop to extract water from the entire soil profile (Wright and Nageswara, 1994).

Awal and Ikeda (2002) reported that chlorophyll concentration, stomatal conductance, photosynthesis and relative growth rate were increased after re-watering (Jogloy et al., 2010). Therefore, of all phenotypic traits that can be altered to improve drought resistance of cereal crops, increased penetration and extension of root systems probably offers the greatest potential (Passioura, 2007). By penetrating deeper into the soil, crop roots potentially access and exploit a greater volume of stored water (McKenzie et al., 2009).

The ability to grow deep roots is currently the most accepted target trait for improving drought resistance, but genetic variation has been reported for a number of traits that may affect drought response. Roots are the principal plant organ for nutrient and water uptake. Therefore, improving our understanding of the interaction between root function and drought in maize could have a significant impact on global food security (Henry et al., 2011).

The effect of selection under stress on yield performance of genotypes under optimal conditions and vice versa has been an ongoing debate among plant breeders for decades. Secondary traits can improve the precision with which drought tolerant genotypes are identified, compared with measuring only grain yield under drought stress. Secondary traits such as canopy temperature, stomata conductance, ears per plant and anthesis silking interval have been found to possess strong correlations with grain yield under drought conditions and have been used to select for higher levels of tolerance to drought (Badu-Apraku et al., 2011). There is therefore a need to evaluate for differences in root, shoot and physiological traits of different maturity groups of maize. Each maturity group of maize has its unique advantages and disadvantages with respect to climatic conditions (e.g. rainfall pattern).

MATERIALS AND METHODS

Two experiments were conducted: Field and pot experiment. Both experiments were conducted at the Research and Teaching Farm of Department of Agronomy, Faculty of Agriculture, Bayero University, Kano (Lat 11°58'N, Long 8°25'E and 475 m above sea level).

The materials used for the experiment were six (6) maize genotypes (Table 1) supplied by the Department of Agronomy, Faculty of Agriculture, Bayero University, Kano. The treatments were laid out in a randomized complete block design (RCBD) with three replications.

Field experiment

Land used for the experiment was ploughed and harrowed to a fine tilt. The farm area was marked out into plots and replications. One ridge was used to represent a plot and each ridge was 4 m long. The seeds were sown manually into their respective ridges at the rate of 2 seeds per hole. The seeds were sown at intervals of 75 × 40 cm inter and intra row spacing, respectively. The plants were thinned to leave one plant per stand at 2 weeks after sowing. Weeding was carried out twice, the first weeding was carried out manually using hoe at 2 weeks after sowing, while the second weeding was carried out using animal traction at 4 weeks after sowing. The recommended dose of fertilizer for maize, 120:60:60 - N: K_2O: P_2O_5 kg/ha, respectively were applied at two weeks after sowing, by side placement. Nitrogen was supplied in two split doses, the first dose at two weeks together with phosphorus and potassium and the second dose at 4 weeks after sowing.

Pot experiment

This experiment was conducted in 30 days. The experiment was conducted in buckets with a volume of 6 L. The buckets were arranged in a complete randomized design with three replications and a bucket representing a plot. The buckets were filled with top soil up to the 4 L mark and were irrigated. The maize varieties were then sown. Weeding and irrigation were carried out routinely.

Statistical analysis

Data collected were analyzed using the PROC MIXED statement. The analysis was done using SAS 9.0 (2001). Replication was considered as a random effect and the genotypes were considered as fixed effect. In the pot experiment, analysis of covariance was performed for all traits using shoot length as a covariate to identify the influence of seedling vigor. Simple correlation among trait was calculated using PROC CORR statement.

RESULTS AND DISCUSSION

Mean performance of genotypes for field experiment

The mean performances of the genotypes for some agronomic traits are presented in Table 2. There was no significant difference (P>0.05) for all the traits, except for days to anthesis, silking and anthesis silking interval (ASI). The variability in these traits is due to differences in the maturity group of the genotype.

Table 3 shows the pair wise comparison for the genotypes for some agronomic traits. There was no significant difference (P>0.05) in the comparison, except for days to anthesis, days to silking and ASI in comparison

Table 1. List of genotypes used for the study.

Entry	Genotype	Maturity	Days to maturity
1	SAMMAZ 14	Late	>110
2	SAMMAZ 29	Extra-Early	80-85
3	2009 EVDT	Early	90-95
4	2009 TZE-W	Early	90-95
5	TZE-COMP 5	Early	90-95
6	2009 TZEE	Extra-Early	80-85

Table 2. Mean performance for some agronomic traits of different maturity group of maize.

Entry	Days to anthesis	Days to silking	Anthesis silking interval	Plant aspect	Plant height (cm)	Ear height (cm)	Leaves number	Yield ($kgha^{-1}$)
SAMMAZ 14	63.00	65.67	2.67	1.67	184.00	63.67	11.33	2022.44
SAMMAZ 29	54.33	60.33	2.00	2.17	171.67	54.67	10.67	1510.46
2009 EVDT	55.33	57.00	1.67	2.00	174.67	56.00	9.67	1965.25
2009 TZE-W	54.67	56.67	2.00	1.83	172.67	60.33	9.67	2291.22
TZE-COMP 5	55.33	61.67	6.33	2.17	177.00	58.00	9.33	2054.39
2009 TZEE	55.00	56.67	1.67	2.00	168.00	50.00	9.67	1891.19
SE ±	1.38	1.71	0.77	0.29	7.46	4.28	0.58	262.48
Genotype	**	*	**	NS	NS	NS	NS	NS

*, ** = significant at 5 and 1% level of probability, respectively. NS = Not significant.

Table 3. Pairwise comparison for some agronomic traits of different maturity group of maize.

Entry	_entry	Days to anthesis	Days to silking	Anthesis silking interval	Plant aspect a	Plant height (cm)	Ear height (cm)	Leaves number	Yield ($kgha^{-1}$)
SAMMAZ 14	SAMMAZ 29	8.67**	5.33*	0.67	-0.33	12.33	9	0.67*	511.98
SAMMAZ 14	2009 EVDT	7.67**	8.67**	1	0.5	9.33	7.67	1.67	57.19
SAMMAZ 14	2009 TZE-W	8.33**	9.00**	0.67	1.00*	11.33	3.33	1.67	-268.78
SAMMAZ 14	TZE-COMP 5	7.67**	4.00	-3.67**	0.17	7	5.67	2.00*	-31.95
SAMMAZ 14	2009 TZEE	8.00**	9.00**	1	0.17	16	13.67*	1.67	131.25
SAMMAZ 29	2009 EVDT	-1	3.33	0.33	0.83	-3	-1.33	1	-454.79
SAMMAZ 29	2009 TZE-W	-0.33	3.67	2.20E-16	1.33*	-1	-5.67	1	-780.76
SAMMAZ 29	TZE-COMP 5	-1	-1.33	-4.33**	0.5	-5.33	-3.33	1.33	-543.93
SAMMAZ 29	2009 TZEE	-0.67	3.67	0.33	0.5	3.67	4.67	1	-380.73
2009 EVDT	2009 TZE-W	0.67	0.33	-0.33	0.5	2	-4.33	-2.78E-17	-325.97
2009 EVDT	TZE-COMP 5	1.00	-4.67	-4.67**	-0.33	-2.33	-2	0.33	-89.13
2009 EVDT	2009 TZEE	0.33	0.33	-1.10E-15	-0.33	6.67	6	2.64E-15	74.06
2009 TZE-W	TZE-COMP 5	-0.67	-5	-4.33**	-0.83	-4.33	2.33	0.33	236.83
2009 TZE-W	2009 TZEE	-0.33	1.42	0.33	-0.83	4.67	10.33	2.66E-15	400.03
TZE-COMP 5	2009 TZEE	0.33	5	4.67**	8.49	9	8	-0.33	163.2
SE ±		1.95	2.37	0.77	0.45	10.56	6.05	0.76	371.21

*, ** = significant at 5 and 1% level of probability, respectively.

of SAMMAZ 14 and other genotypes, where there was significant difference (P<0.01). There was a highly significant difference (P<0.01) in comparison among TZEE-COMP 5 and other genotypes for ASI. This variation

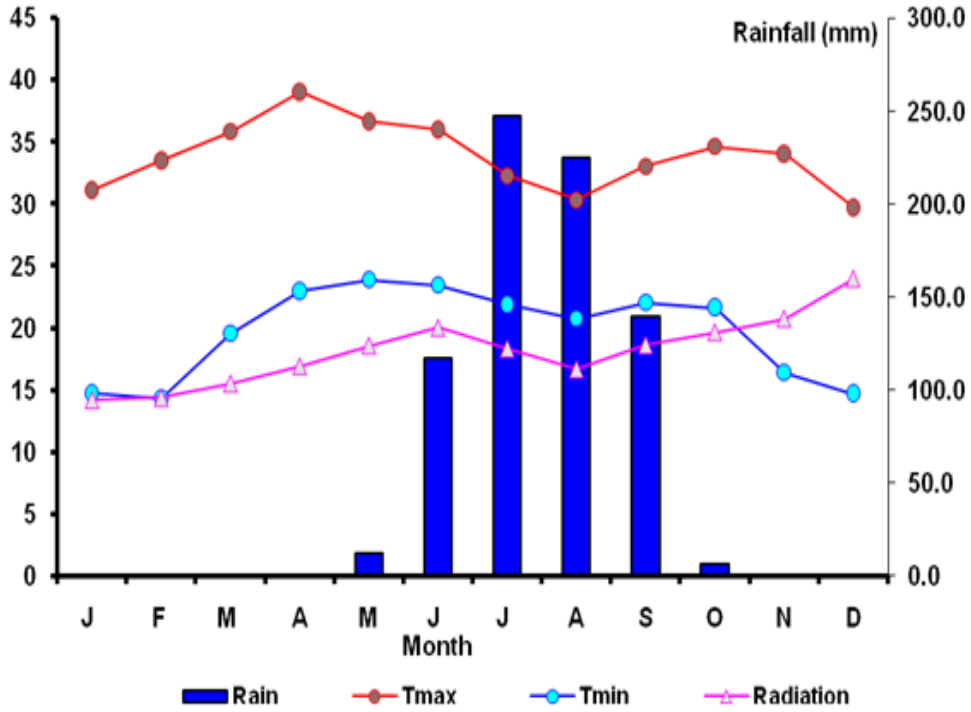

Figure 1. Meteorological data of the experimental area in 2014.

Table 4. Mean performance for some physiological traits of different maturity groups of maize.

Entry	Leaf temperature (°C)	Canopy temperature (°C)	Stomatal conductance (mmd/m^{2}s)	Chlorophyll content (SPAD)
SAMMAZ 14	32.87	29.23	1859.67	45.48
SAMMAZ 29	32.77	29.37	2533.07	44.17
2009 EVDT	32.40	28.00	2366.47	49.37
2009 TZE-W	32.60	28.87	1880.33	42.78
TZE-COMP 5	32.40	28.20	2092.53	42.25
2009 TZEE	32.60	27.57	2104.00	49.28
SE ±	0.34	1.07	340.02	3.09
Genotype	NS	NS	NS	NS

NS = Not significant.

also reflects the difference in the maturity groups of the genotypes and also classifies the genotypes into their respective groups. Also the lack of significant difference for other traits measured can be due to low rainfall (water stress) observed during the experimental period (Figure 1).

The mean plant height and ear height are within reasonable range compared with the report of Menkir and Akintunde (2001). The mean anthesis - silking interval (ASI) was 3 days for the genotypes. The shortened ASI observed in these cultivars is desirable because it has been reported that low ASI enhance maize tolerance to stresses during flowering and it ensures good grain filling (Edmeades et al., 1993; Bolanos and Edmeades 1996).

The mean performances of some physiological traits are presented in Table 4. There was no significant difference ($P>0.05$) among genotypes for all the traits measured. The lack of significant difference observed in the physiological traits of the genotypes measured was indications that irrespective of the difference in maturity group the physiological response of the maize genotypes are the same. This also confirms that variability in the traits does not exist between the different maturity groups of the maize genotypes. The maize genotypes showed a good response in terms of improving them towards becoming drought tolerant genotypes.

Table 5. Mean squares for some root and shoot traits of different maturity group of maize in screen house.

SOV	df	Dry root shoot ratio	Dry root weight (g)	Dry shoot weight (g)	Fresh root shoot ratio	Fresh root weight (g)	Fresh shoot weight (g)	Leaves number	Root length (cm)
Entry	5	0.22	43.23	4.88	0.41	220.46	138.45	0.22	10563
Covariate	1	1.74**	689.08**	154.75**	0.22	6886.51**	7117.46**	37.62**	42917
Residual	11	0.09	28.70	5.44	0.33	62.14	78.59	0.28	6826

*, ** = significant at 5 and 1% level of probability, respectively.

Table 6. Mean performance for root and shoot traits of different maturity group of maize in screen house.

Genotype	Dry root shoot ratio	Dry root weight (g)	Dry shoot weight (g)	Fresh root shoot ratio	Fresh root weight (g)	Fresh shoot weight (g)	Leaves number	Root length (cm)
SAMMAZ 14	0.61	3.49	5.31	0.54	10.46	22.68	6.28	28.10
SAMMAZ 29	1.21	6.61	4.91	0.83	20.22	19.89	6.93	70.43
2009 EVDT	1.41	13.46	6.09	0.96	29.86	26.03	6.68	187.16
2009 TZE-W	0.94	2.69	2.84	0.92	11.33	11.81	6.28	38.10
TZE-COMP 5	1.07	6.53	6.30	1.66	29.99	31.78	6.38	34.25
2009 TZEE	0.91	5.88	5.89	0.94	17.93	18.35	6.79	84.30
SE $\pm$	0.26	4.43	1.93	0.48	6.52	7.34	0.44	68.40
Genotype	NS	NS	NS	NS	NS	NS	NS	NS

NS = Not significant.

Mean performance of genotypes for pot experiment

The mean performances of the genotypes for some agronomic traits are presented in Table 5. There was no significant difference (P>0.05) among the genotypes for all the traits measured. There was significant difference (P<0.01) among the genotypes with respect to the shoot length (covariate) for all the traits measured, except for fresh shoot root ratio and root length. This indicated that seedling vigour was detected. Shoot length had effects on the fresh and dry shoot weight, fresh and dry root shoot ratio, as well as, dry root weight. The lack of significant covariate difference observed for fresh root shoot ratio and root length shows that the differences in the maturity group of the genotypes does not affect these traits.

Table 6 shows the mean performance of the genotypes for some agronomic traits. There was no significant difference (P>0.05) among the genotypes for all the traits measured. No significant difference was observed among the genotypes for the agronomic traits measured at seedling stage. This shows that the genotype has similar pattern of root and shoot development. However, 2009 EVDT a drought tolerant variety gave a better response in terms of root development response followed by Sammaz 29. This is an indication that Sammaz 29 can be improved to be drought tolerant.

Correlation among some agronomic traits of the genotypes is presented in Table 7. Most of the correlation among the traits showed no significant relationship (P>0.05). There was positive and highly significant (P<0.01) correlation in the correlation among leaf temperature with fresh root weight (0.64), fresh shoot weight (0.65), and dry shoot weight (0.64); and with dry root weight (0.50) and shoot length (0.64) at P<0.05. This shows that a change in leaf temperature will result in a change in these traits. Also root traits were found to have positive effect on yield. Results obtained are in accordance with those reported by Khan et al. (2002), Dhanda et al. (2004), Awan et al. (2007) and Rauf et al. (2007). As root-shoot ratio was negatively correlated with some traits so selection for low root-shoot ratio will decreases the performance of other important seedling traits (Khan et al., 2010). Similar results have also been reported earlier by Echarte and Tollenaar (2006) and Ojo et al. (2006).

A high correlation was observed between plant and ear height. A high correlation between plant height and ear height has been reported by Hallauer and Miranda (1995), Nato and Miranda (2001), and Salami (2002). The close relation among these traits will cause them to respond similarly during improvement.

Conclusion

Drought, which is a rising threat of the world, can be adapted to with genotypes with efficient root system. Improvement in root and physiological traits of maize genotypes can lead to improvement in level of tolerance

Table 7. Correlation matrix of some physiological, root and agronomic traits of maize.

Variables	Plant apect	Canopy temp.	Leaf temp.	Stomatal conductance	Chlorophyll content	Plant height	Ear height	Yield	Root length	Fresh root weight	Dry root weight	Shoot length	Fresh shoot weight	Dry shoot weight	Fresh root shoot ratio	Dry root shoot ratio
Plant aspect	1															
canopy temperature	0.29	1														
Leaf temperature	-0.15	-0.29	1													
Stomatal conductance	-0.04	0.26	-0.09	1												
Chlorophyll content	-0.21	-0.18	-0.31	0.29	1											
Plant height	-0.27	-0.21	0.23	0.14	0.31	1										
Ear height	-0.37	-0.10	0.19	0.05	0.14	0.91**	1									
Yield	-0.56*	-0.27	0.17	0.15	-0.02	0.51*	0.51*	1								
Root length	-0.09	0.25	0.17	0.17	-0.09	-0.17	-0.16	-0.01	1							
Fresh root weight	-0.52*	-0.31	0.64**	-0.05	0.22	0.33	0.24	0.23	0.07	1						
Dry root weight	-0.55*	-0.30	0.50*	0.10	0.36	0.32	0.21	0.10	-0.04	0.92**	1					
Shoot length	-0.54*	-0.25	0.64*	-0.24	0.04	0.28	0.23	0.33	0.05	0.90**	0.76**	1				
Fresh shoot weight	-0.47*	-0.40	0.65**	-0.17	0.18	0.38	0.31	0.28	-0.07	0.95**	0.82**	0.92**	1			
Dry shoot weight	-0.45	-0.52*	0.64**	-0.17	0.31	0.44	0.31	0.14	-0.09	0.89**	0.86**	0.82**	0.91**	1		
Fresh root shoot ratio	-0.04	0.12	0.12	0.21	0.08	0.24	0.15	-0.11	0.44	0.08	0.12	-0.15	-0.09	0.09	1	
Dry root shoot ratio	-0.52*	-0.04	0.41	0.26	0.22	0.17	0.07	0.05	0.19	0.82**	0.89**	0.67**	0.67**	0.62**	0.26	1

*, ** = significant at 5 and 1% level of probability, respectively.

to drought. The genotypes used in this study shows a good response to drought and no variability was observed between the genotypes for the root and physiological traits observed. This is an indication that maturity period did not influence the response of maize to these traits as such maize has similar response pattern to root and physiological traits. Leaf and canopy temperature has relationship with root weight, shoot weight and leaf number. This shows root characteristics can be improved by increasing the leaf temperature. The existing correlation between leaf temperature and root system is an important factor which breeders should consider in production of these genotypes.

The lack of significant difference in the physiological traits of the genotypes which included a drought tolerant genotype (2009 EVDT) shows that these genotypes can also be improved to become drought tolerance genotypes. Also irrespective of maturity group of the maize, their root and physiological responses are the same.

Conflict of Interest

The authors have not declared any conflict of interest.

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